THE RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE BIOLOGICAL ACTIVITY, AFFINITY, AND EFFICACY IN COMPOUNDS RELATED TO ACETYLCHOLINE

by

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Contents

Part I

INTRODUCTION

The Mode of Action of Drugs

The word "drug" can be used to describe any molecule which modifies, in any way whatsoever, the physical and chemical processes occurring in normal tissues. At a molecular level it is possible that this interference may be the result of the chemical properties of the drug, or of its physical properties, or of a combination of the two. For example, A.J. Clark (1933, 1937) calculated that the number of molecules of acetylcholine which produced detectable slowing of the rate of beating of the frog's ventricle was so small that only a small fraction of the total area of the cells would be covered with molecules of the drug. The number of molecules of caffeine, however, which was needed to produce a detectable effect was much larger and should suffice to cover the whole area of the cells.

Such calculations are evidence for the idea that some drugs act by affecting "receptors", particular groups in the tissues which occupy only a small part of the cell surface. This idea was originally put forward by Langley (1878, 1905) and was used extensively by Ehrlich (1913) to interpret results in his work on Chemotherapy. The idea is particularly attractive because of subsequent discoveries in heterogeneous catalysis and in enzymology. In some instances, e.g. eserine, fluoracetate, it can be shown that the pharmacological effects of a drug are directly related to its ability to block, specifically, a particular enzyme and that the receptors with which the drug combines to produce its effects are the "active spots" on the enzyme.

It cannot always be assumed that the receptors are active spots on an enzyme ; indeed it may be misleading to do this ; but even if the receptor is regarded only as a hypothetical entity,

it is possible to apply the Law of Mass Action to the reaction between drug and receptor after the manner of Langmuir (1916, 1918). Let the reaction be written:-

Drug + Receptor = Complex Breakdown Response

The drug is here called an agonist because it is capable of producing the biological response. For a concentration of drug [A], the rate of formation of the complex will be $k_1 A\ (1-y)$, where y is the proportion of receptors which are combined with the drug. The rate of breakdown of the complex will be $k_2 y$. At equilibrium the rates will be equal so it is possible to write

$$K_a A = \frac{y}{1 - y} \qquad (1)$$

where K_a is the association constant for the formation of the complex. This expression, derived by Clark (1933), is very similar to that used by Michaelis and Menten (1913) relating the concentration of substrate to the proportion of active spots occupied on an enzyme except that Michaelis and Menten used the dissociation constant K_M for the complex i.e.

$$K_M = 1/K_a \qquad (2)$$

If a second substance, B, is present which is capable of combining with the receptors but not of causing the biological response and is, therefore, an antagonist, the rate of formation of the complex with A the agonist will be $k_1 A\ (1-y-z)$ and the rate of breakdown $K_2 y$ so

$$[A]K_a = \frac{y}{1- y - z} \qquad (3)$$

for the antagonist drug B,

$$BK_B = \frac{z}{(1 - y - z)} \qquad (4)$$

where K_B is the association constant for the antagonist and the receptor.

From this it can be shown that

$$AK_A = \frac{y}{1-y} (1 + BK_B) \qquad (5)$$

In the breakdown of a substrate by an enzyme the value, y, may be directly related to the rate of the reaction (V) because it is supposed that the breakdown of the complex to give the products is the rate-determining step.

$$V = k\, y, \qquad (6)$$

where k is the rate constant for the reaction. When $y = 1/2$, the velocity of the reaction should be $1/2\ V_{Max}$, where V_{Max} is the maximum rate obtainable i.e. when $y = 1$ and the enzyme is saturated. It follows, then that

$$A/K_M = 1, \qquad (7)$$

and this technique for measuring K_M by determining the substrate is frequently employed in Enzymology (Dixon and Webb, 1959).

In pharmacological reactions, however, the situation is different because the steps between the formation of the complex and the biological response are completely obscure. If the adsorption of the drug obeyed the Langmuir Isotherm, and if the response varied directly with y, then the concentration of A for which the response was half-maximal would be equal to $1/K_a$ and hence a fundamental constant. This has often

been assumed that the p_D values, used by Miller, Becker and Tainter (1948) and by Ariens *et al.* (1957) are the logarithm of the reciprocal of the concentration producing half the maximal response and are supposed to represent the logarithm of the affinity constant of the drug and the receptors.

There are two assumptions involved which are difficult to justify, that adsorption follows the Langmuir isotherm and that the biological response is directly related to the proportion, y, of receptors occupied. Clark (1933, 1937) pointed out that it was not possible to test the validity of the relationship

$$A K_a = \frac{y}{1 - y} \qquad (8)$$

by experimental methods. The inaccuracies inherent in measuring the response are such that the results could equally well be interpreted on the basis of an adsorption of the Freundlich type,

$$y = K A^{1/2} \qquad (9)$$

or according to the empirical Weber-Fechner "Law"

$$Ky = \log (bA + 1) \qquad (10)$$

In spite of advances in experimental techniques Clark's remarks are still applicable today. It is possible to justify the use of the Langmuir adsorption Isotherm on the grounds that it can be applied successfully in physical chemistry and in enzymology but the chief reason for its application to drugs and receptors is that it is a simple and reasonable explanation which is not incompatible with the experimental results. The "rate" theory proposed by Paton (1961) is another such theory which may also be regarded as being not incompatible with the experimental results but fortunately it

leads to a similar relationship between the drug concentration, A, and the proportion, y.

The assumption that this proportion, y, of the receptors occupied can be related directly to the biological response is much more difficult, if not impossible to justify. Some drugs act purely as antagonists e.g. atropine at postganglionic cholinergic receptors in the autonomic nervous system or (+)-tubocurarine at the neuromuscular junction. Even when the receptors are saturated with such drugs there is absolutely no biological response. From experiments with acetylcholine and tetramethylammonium on the frog rectus, Clark and Raventos (1937) thought it possible that ability to stimulate receptors might be an all-or-none property. Although the concentration of tetramethylammonium which caused contracture of the rectus was about 1,000 times that of acetylcholine, these compounds acted additively i.e. if a concentration A of acetylcholine produced the same effect as a concentration T of tetramethylammonium, then the same effect was also obtained with A/2 + T/2. If tetramethylammonium were less able to stimulate the receptors than acetylcholine, it would be expected that the presence of the tetramethylammonium would reduce the number of receptors available for combination with acetylcholine and hence the two compounds would not act additively and the response to A/2 + T/2 would be less than to A or T alone. From the results of Clark and Raventos it appeared that the lower activity of tetramethylammonium compared with acetylcholine could be ascribed simply to a lower affinity for the receptors.

This situation cannot be extended

to all compounds which are capable of acting as agonists. Stephenson (1956) and Ariens and de Groot (1954) have shown that there are other compounds, alkyltrimethylammonium salts, for instance, which act like acetylcholine on the guinea-pig ileum or frog rectus but which do not act additively with acetylcholine. With some of them it is even impossible to cause a maximal contraction of the tissue however high the concentration of the compound. These compounds have been termed "partial agonists" or "dualists" and to account for their properties Stephenson has suggested that the biological response to a particular concentration of antagonists depends not only upon y, the proportion of receptors occupied, but also on a function e, the efficacy, which may vary from compound to compound (Ariens *et al.* use the symbol α which they call intrinsic activity). Stephenson thus writes:-

The response = some function of S,

where $S = ey.$ (11)

According to Stephenson e may have any value from zero upwards, but it is difficult to measure for a substance which is a pure agonist. It is not sufficient to measure the pD value because the nature of the function relating biological stimulus to response is unknown. If the two were directly related, then from the equation (11), it should follow that the value e would be given by the ratio of the size of the maximal response which could be produced by the drug to the maximal response of which the tissue was capable of producing in response to a substance such as acetylcholine. This ratio is, in fact, used by Ariens *et al.* to compute the value of the intrinsic activity-

which must accordingly lie between zero and unity - but it may be questioned whether it is any real measure of efficacy. Not only is it unjustified to assume that the biological response is directly related to the biological stimulus but it is also questionable whether it is necessary to have all the receptors occupied in order to obtain a maximal response. Most biological processes appear to operate with a considerable "safety factor" and it is quite conceivable that a maximal response could be produced by the combination by a drug of high efficacy with only a small proportion of the total receptor population. Consequently a maximal response could be produced by compounds with a considerably lower efficacy than others. For example, if only 10% of the receptors combine with acetylcholine and give rise to a maximal response, a compound with an efficacy of only 10% of that of acetylcholine would still appear to have an "intrinsic activity" of 1, (see page 10)

To compare the activity of two agonists it is usual to measure the concentrations which produce comparable effects:
in these circumstances

$$S = e_1 y_1 = e_2 y_2$$

or

$$\frac{e_1 K_1 A_1}{1 + K_1 A_1} = \frac{e_2 K_2 A_2}{1 + K_2 A_2} \qquad (12)$$

If the absolute values of A_1, A_2, K_1 and K_2 were known it should be possible to estimate the ratio e_1/e_2. The absolute values of the affinity constants of pure agonists are unobtainable (they depend upon knowning e_1 and e_2) but they are easily obtainable for antagonists and Stephenson has

devised a method for obtaining the values for partial agonists which do not produce a maximal response.

The equation $AK_a = \frac{y}{1-y}(1 + BK_b)$ (13)

connects the concentrations of agonist, antagonist, affinity constants and proportion of receptors occupied by agonist. If a concentration a of agonist produces, in the absence of any antagonist, a response which is the same as that produced by a (bigger) concentration A in the presence of a concentration B of antagonist, we can write

$$aK_a = \frac{y}{1-y} \quad (14)$$

and $$AK_a = \frac{y}{1-y}(1 + BK_b) \quad (15)$$

but as the response is the same in each experiment, y should be the same and hence the "dose ratio"

$$A/a = (1 + BK_b) \quad \text{(Gaddum, 1937)} \quad (16)$$

The value of K_b can, accordingly be obtained from the graph of the dose ratio minus one against the concentration of antagonist, or alternatively by measuring the concentration of B for which the dose-ratio is 2. The latter is the technique used by Schild (1947, 1949) who calls the logarithm of the reciprocal of this concentration pA_2. This is actually the value of log K_b.

To obtain estimates of K for a partial agonist it is necessary to suppose that the proportion of receptors occupied is small, consequently AK_a will approach y [since $(1 - y) \rightarrow 1$] and S will approximate to eKA. Then let an experiment be performed in which the response (I)

to a concentration P of a partial agonist (efficacy e_p) is matched by a concentration A_1 of an active agonist (efficacy e_a, affinity constant K_a), and a response (II) to P plus A_3 of agonist is matched by A_2 of agonist alone (Fig.). If the partial agonist occupies a proportion x of the receptors, the response I is produced by a stimulus

$$S_1 = e_p x = e_a K_a A_1 \qquad (17)$$

The response II is produced by a stimulus

$$S_2 = e_a K_a A_2 = e_p x + e_a K_a A_3 (1-x) \qquad (18)$$

assuming that values of S are additive and that the active agonist occupies only a negligible proportion of receptors, whereas the partial agonist occupies a significant proportion.

Then,

$$e_a K_a A_2 = e_a K_a A_1 + e_a K_a A_3 (1-x)$$

or

$$I - x = \frac{A_2 - A_1}{A_3} \qquad (19)$$

Values of x for different concentrations of partial agonist can be obtained and the true concentration producing 50% occupation of the receptors determined.

Values of the affinity constant, K, obtained in this way are not the same as those obtained from the concentration producing half the maximal response. For instance, Stephenson found the affinity constant for n-heptyltrimethylammonium on the guinea-pig ileum at 37° to be 4.1×10^4, whereas the concentration giving half the maximal response of which the drug was capable was 5×10^{-6} m, which should give an affinity constant of 2×10^5.

Calculations of this type assume the existence of spare receptors and evidence for this concept has been obtained from two types of experiment. Stephenson (1956) observed that in the alkyltrimethylammonium salts, the affinity of those which were partial agonists appeared to vary in geometrical progression with increasing chain length. If it is assumed that the variation of affinity with chain length could also be extended to the lower members which were agonists, the extrapolated values for their affinity constants were much higher than the concentrations producing 50% response, i.e. less than 50% of receptors were occupied by concentrations which gave a 50% response.

Other evidence has been obtained by using non-equilibrium (irreversible) blocking agents (Stephenson, 1956; Nickerson, 1956 and Ariens, van Rossum and Simonis, 1956). In their experiments the log. dose-response curve for the agonists is determined before and after the preparation has been treated with the blocking agent. When only a small degree of block is produced, the log. dose-response curves are parallel to the original, but after more extensive block the agonist can no longer produce a maximum response. In the absence of spare receptors the size of the maximum response would be reduced by even mild treatment with this kind of blocking agent.

Aim of the present work

The present work describes an attempt to study the effects of changes in chemical structure on the affinity and efficacy of compounds related to acetylcholine. Although the absolute measurement of either of these properties is extremely difficult in principle for agonists, Stephenson suggested that if two series of compounds were prepared, one purely antagonist and the other agonist, changes in affinity with structure could easily be measured in the series of antagonists, and might be applicable to corresponding agonists. From these, and from the experimentally determined changes of activity with structure it might be possible to deduce something about the changes of efficacy with structure.

Compounds related to acetylcholine seemed to be the simplest to study, the series of agonists containing an intact acetyl or methyl group and the series of antagonists being the analogous diphenylacetyl, or diphenylmethyl derivatives. Some benzilyl derivatives were also prepared.

From the theory of Arrhenius, the relationship between the association constant, K, for the drug and receptor, and the free energy change on adsorption (ΔF) is

$$\Delta F = -RT\log_e K \qquad (20)$$

or

$$\log_{10} K = \frac{-\Delta F}{2.3RT} \qquad (20a)$$

In the series

$R\overset{+}{N}Me_3$	$R'\overset{+}{N}Me_3$
$R\overset{+}{N}Me_2Et$	$R'\overset{+}{N}Me_2Et$
$R\overset{+}{N}MeEt_2$	$R'\overset{+}{N}MeEt_2$
$R\overset{+}{N}Et_3$	$R'\overset{+}{N}Et_3$

let it be assumed that the change in the free energy of adsorption is only dependent on the substitution in the onium group, i.e. that the free energy of adsorption is made up of components which are additive, the contribution from the portion R (whatever it may be) being unaffected by changes in the onium group: this implies that there is no interaction between R and the individual substituents on the quaternary nitrogen atom. We can then write, if the free energy of adsorption for $R\overset{+}{N}Me_3$ is ΔF, and for $R'\overset{+}{N}Me_3$ $\Delta F'$, the free energy of adsorption of

$R\overset{+}{N}Me_2Et$	$= \Delta F + a$	$R'\overset{+}{N}Me_2Et$	$= \Delta F' + a$
$R\overset{+}{N}MeEt_2$	$= \Delta F + b$	$R'\overset{+}{N}MeEt_2$	$= \Delta F' + b$
$R\overset{+}{N}Et_3$	$= \Delta F + c$	$R'\overset{+}{N}Et_3$	$= \Delta F' + c$

where "(a)" is the free energy change brought about by replacing $\overset{+}{N}Me_3$ by $\overset{+}{N}Me_2Et$, "(b)" the change for replacing $\overset{+}{N}Me_3$ by $\overset{+}{N}MeEt_2$ and "(c)" the change for replacing $\overset{+}{N}Me_3$ by $\overset{+}{N}Et_3$.

The value of K for the series of antagonists have been determined experimentally; in the series $R\overset{+}{N}Me_3$, $R\overset{+}{N}Me_2Et$ etc., let these be K, K_a, K_b and K_c. It should then follow that

$$\log K = \frac{-\Delta F}{2.3\ RT} \qquad (20a)$$

$$\log K_a = \frac{-(\Delta F + a)}{2.3\ RT} \qquad (20b)$$

therefore,

$$\log K_a/K = \frac{-a}{2.3\ RT} \qquad (21)$$

Values can similarly be obtained for "b" and "c". These values should be the same regardless of the nature of R, and this can be investigated

by using more than one series of antagonists.

In the series of agonists, $R\overset{+}{N}Me_3$ etc., the absolute value of the affinity or the free energy of adsorption cannot be determined in these experiments but the change in the free energy of adsorption produced by altering the cationic head should be the same, a, b and c, as in the series of antagonists. Suppose that the two compounds $K\overset{+}{N}Me_3$ and $R\overset{+}{N}Me_2Et$ have affinity constants K and K_a and that the equipotent molar ratio for $R\overset{+}{N}Me_2Et$ relative to $R\overset{+}{N}Me_3$ is n, i.e. n molecules of the $R\overset{+}{N}Me_2Et$ are needed in order to produce the same response as one molecule of $R\overset{+}{N}Me_3$. Then the stimulus,

$$S = \frac{e\,K\,A}{1 + KA} \qquad (22)$$

$$= \frac{e_a K_a A_a}{1 + K_a A_a}$$

where e is the efficacy of $R\overset{+}{N}Me_3$ and A the concentration producing the response, and e_a the efficacy of $RNMe_2Et$ and A_a the concentration producing the same response: the value A_a/A will be n.

If the proportion of receptors occupied by the drug is relatively small, the expression $KA = \frac{y}{1-y}$ will approximate to y and hence the stimulus

$$S = ey = e\,AK \qquad (23)$$

The expression above then becomes $e\,K\,A = e_a K_a A_a$ and hence the ratio of the efficacies,

$$e/e_a = \frac{K_a A_a}{KA} = \frac{K_a}{K}\quad n. \qquad (24)$$

But the ratio

$$K_a/K = 10^{-\left[\frac{a}{2.3\ RT}\right]} \qquad (25)$$

which has been determined in the experiments with the antagonists. The value of (n), the equipotent molar ratio, has been determined experimentally and hence the ratio can be calculated.

Nomenclature: Throughout this thesis the final products employed in the pharmacological work are referred to by code names constructed from the following abbreviations:

A = acetyl	B = butyryl
E = ethyl	H = hydroxy
M = methyl	O = oxy
P = propyl	Ø = phenyl.

The code name or group is built up by substituting the above symbols for the corresponding constituent parts of a convenient systematic name for the compound. Thus the group $Ø_2$HAOE/M_2E refers to the compound [diphenylhydroxyacetoxyethyl]-dimethylethylammonium (I) and the group MOP/E_3 to the compound [methoxypropyl]-triethylammonium, (II).

$Ph_2C(OH)CO.O.CH_2CH_2\overset{+}{N}(Et).Me_2$ (I)

$CH_3O.CH_2CH_2CH_2\overset{+}{N}.Et_3$ (II)

In each of the "series" referred to herein the principal group, e.g. $Ø_2$HAOE and MOP in (I) and (II) above, is kept constant while the remaining substituents on the quaternary nitrogen are varied from M_3 through M_2E and ME_2 to E_3.

In almost all the compounds the anion is bromide. In the few cases where the bromide could not be prepared the nature of the anion is clearly stated.

Part II

PHARMACOLOGY

PHARMACOLOGY - Experimental

Preparations

The original isolated preparation of Magnus (1904) employed the ileum of the cat. All experiments recorded in this thesis were performed on the modified guinea-pig ileum preparation described by Stephenson (1956).

A guinea-pig, usually female weighing between 150 and 250 g., was killed by a blow on the head and bled. About 10 cm. of ileum was carefully dissected out and placed in a dish of Tyrode solution at room temperature. The lumen of the gut was washed through with Tyrode employing the minimum hydrostatic pressure necessary, usually equivalent to 2-4 cm. water. The terminal 3-4 cm. of the ileum was suspended in Tyrode solution in a bath of 2.7 ml. capacity at 37.0 $\pm$ 0.1. It was attached to a light (total weight excluding load = 2.5 g.) isotonic frontal writing lever giving a magnification of 4 and with a load of 0.5 g.

The bath was connected to coils of glass tubing so that the fluid in the bath could be changed by upward displacement and overflow, either by Tyrode alone or by Tyrode containing drugs at pre-determined concentrations. The coils were of such volume that sufficient solution could be run through the organ bath to effect a complete change without exposing the muscle to air and without cooling (less than 0.1° C.). Events in the bath were controlled by an automatic apparatus similar to that described by Schild (1947).

The Tyrode solution used in all experiments contained double the concentration of potassium and 1.1×10^{-4} molar hexamethonium (equivalent to 40 mg. of the bromide per litre).

Preliminary experiments indicated that increasing the K^+ content of the Tyrode could reduce the magnitude of the "stair-case" effect (Fig. 1, p. observed on changing the concentration of agonist to which the ileum was exposed. The presence of hexamethonium served to reduce the spontaneous activity of the gut with the result that a more satisfactory base-line was obtained.

Methods

Antagonists.- Antagonist activity was estimated by determining the affinity constant (K) of the drug for the receptors in the guinea-pig ileum at 37° C. Originally it was intended to do this merely by measuring pA_2 by the method of Schild (1947), but it became apparent that, as the most accurate possible estimates of the affinity constant would be required in this work, it would be better to use a wider range of concentrations of antagonists than that employed in the determination of pA_2. The following procedure was adopted: within minutes of placing the ileum in the organ bath it was exposed to a concentration of acetylcholine (usually 4×10^{-8} molar) for 10 secons which was then washed away with about 15 ml. of Tyrode. This was repeated at regular intervals of between 60 and 90 seconds until the responses became steady, usually after about an hour. The log. dose-response curve for the agonist was determined using three concentrations of agonist which produced roughly between 20 and 80% of the maximal response (Figs. 2a and 2b, p. Each concentration of agonist was applied to the ileum for 4 or 5 contractions before being replaced by another. The order of application of the various concentrations was

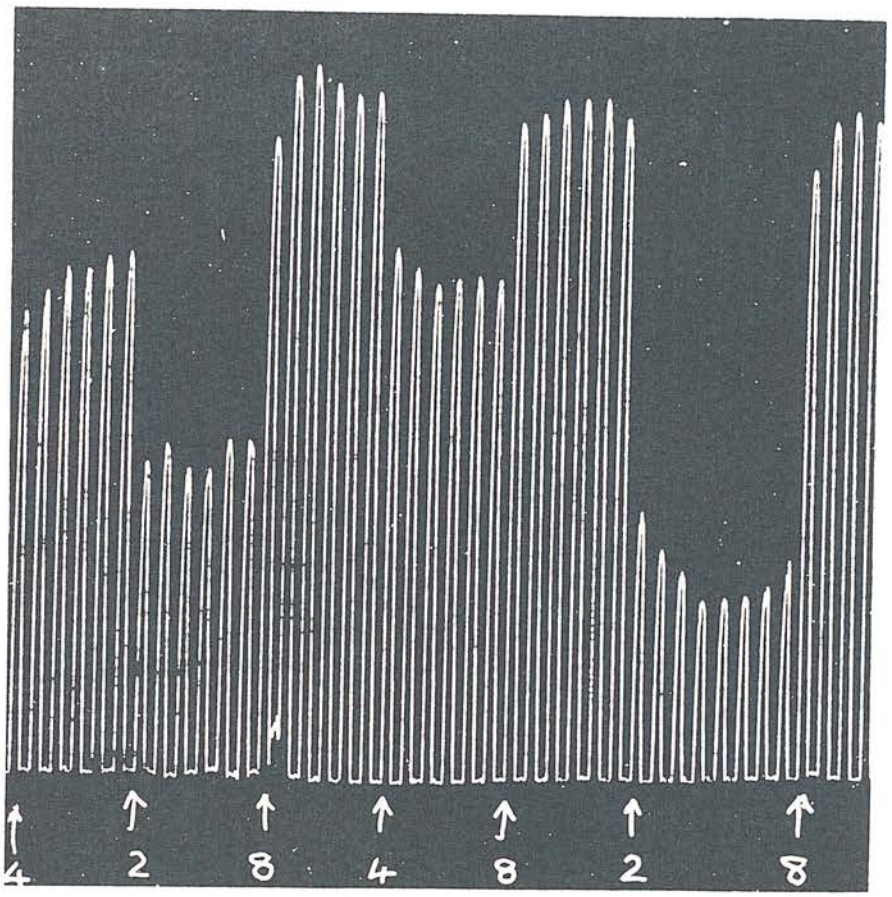


Figure 1. Response of guinea-pig ileum preparation to alteration of dose of acetylcholine (10^{-8} molar) clearly showing the "stair-case" effect frequently encountered in experiments using Tyrode with a normal potassium content.

The use of Tyrode containing double the concentration of potassium has been observed to reduce or even abolish this effect.

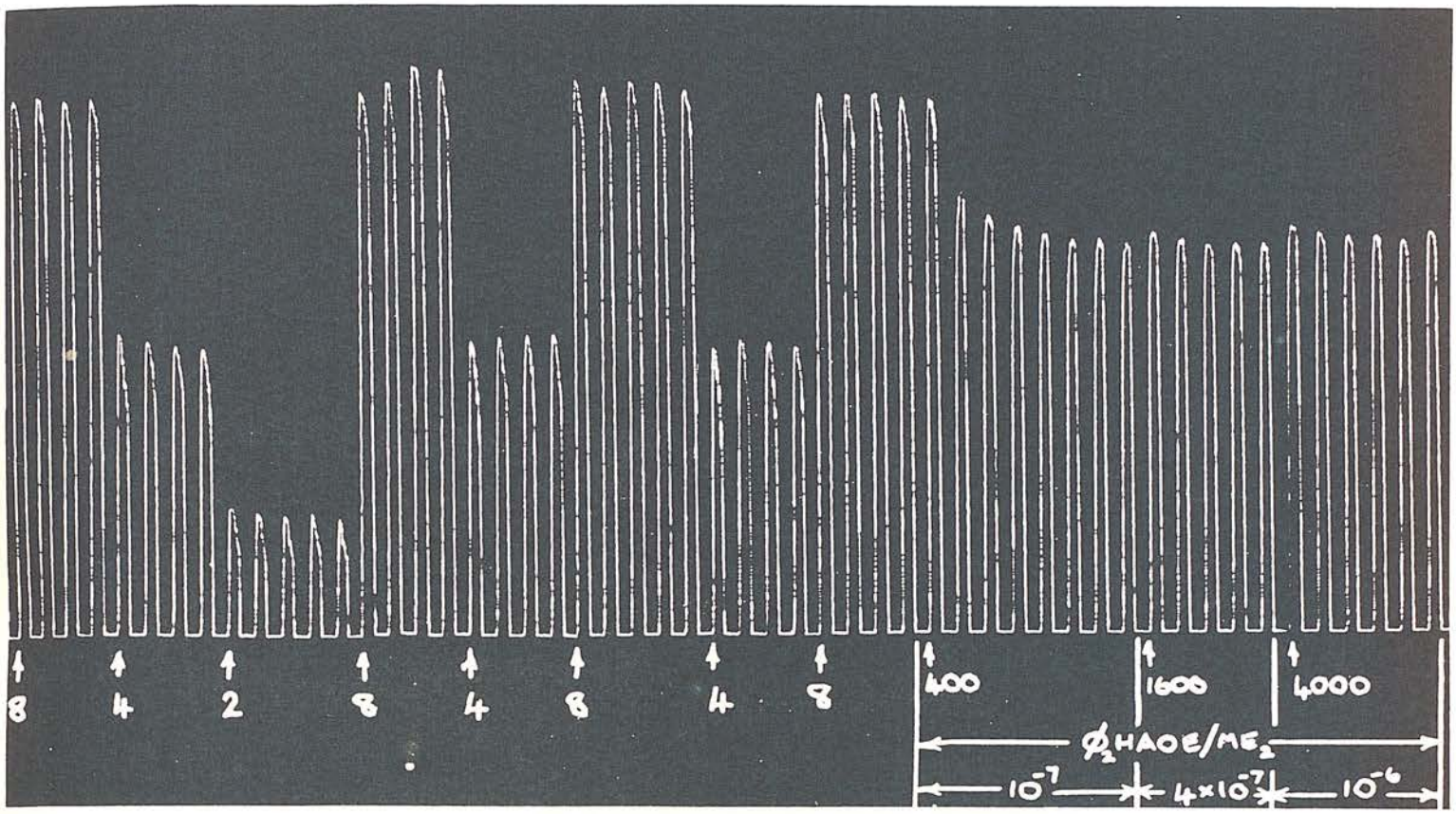


Figure 2(a) Typical antagonist assay showing method of determining (log) dose response curve for agonist prior to application of antagonist.

$\emptyset_2$ MOP/M_3

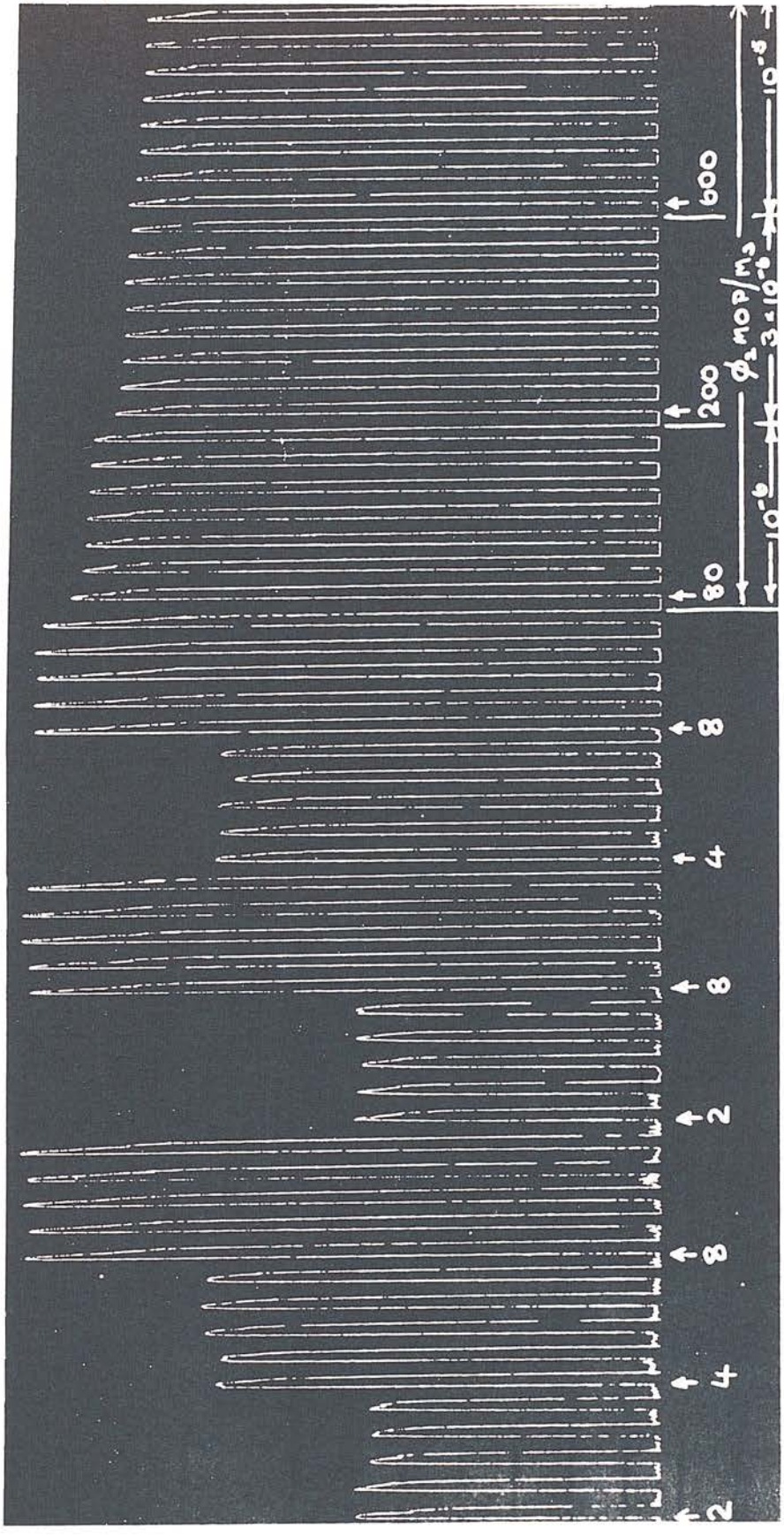


Figure 2(b). Typical Affinity Constant Determination. Drug Contact 10 sec., Cycle 60 sec. (see also Figs.3 and 4)

varied but was always arranged to terminate with successive applications of high, intermediate and high concentrations immediately prior to application of the antagonist. After consistent values had been obtained for the heights of contraction produced by the three agonist concentrations, the Tyrode in which the ileum was suspended (and with which it was washed) was replaced by Tyrode containing the antagonist. At the same time the agonist solution was replaced by another containing a much higher concentration of acetylcholine together with the same concentration of antagonist as was present in the washing Tyrode. The concentration of acetylcholine in the latter solution was calculated, on the basis of preliminary experiments to produce a contraction, in the presence of the antagonist at the concentration employed, equal to or slightly greater than that produced by the intermediate concentration of acetylcholine before application of the antagonist.

The solution containing both agonist and antagonist was applied to the ileum using the same cycle as before, until about five or more consistent contractions were obtained, i.e. until equilibrium was attained. A higher concentration of antagonist was then used and stimulation of the muscle was effected by a correspondingly increased concentration of acetylcholine. In general, as soon as five fairly consistent (equilibrium) contractions had been obtained the antagonist and agonist-antagonist solutions were replaced by ones of higher concentration so that the responses produced in the presence of three or even four different antagonist concentrations could be determined in the shortest possible time after

"standardising" the preparation, i.e. after obtaining consistent values for the responses in the absence of the antagonist.

The equilibrium values of the heights of contraction before application of the antagonist were determined by eye, measured to the nearest 0.5mm. and plotted against the log. concentration of agonist producing the effect. The dose ratio for a particular concentration of antagonist was determined by comparing the size of the (equilibrium) response with that obtained in the absence of the antagonist; if the graph of the log. dose (agonist) - response was not linear, a straight line was drawn joining the two points between which lay the response produced in the presence of the antagonist (Figure 3).

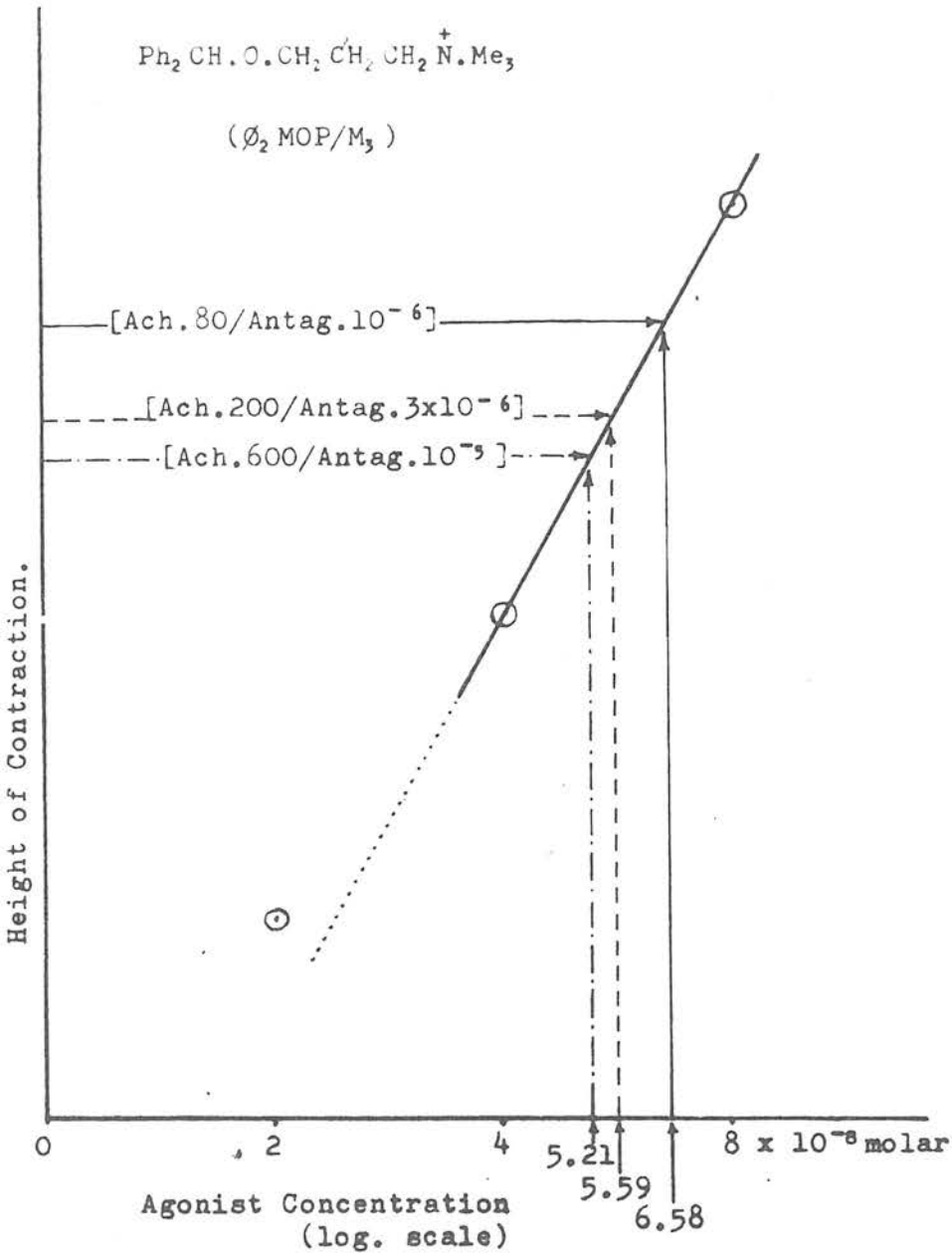
From these results a graph of (dose ratio - 1) against concentration could be plotted and this should be linear if the antagonism is competitive. In all cases in which this was done a linear relationship was in fact obtained; an example is shown in Figures 4 and 5. The affinity constant could be calculated from the slope of this line but was usually estimated for each particular concentration of antagonist by substituting the observed value for the dose ratio in the Gaddum equation:

$$A/a = (1 + BK_b) \qquad (16)$$

or

$$(A/a - 1) = BK_b$$

where A/a is the dose ratio which has been determined: B, the antagonist concentration, is known and hence K_b, the affinity constant, may be evaluated. The determination of K_b was repeated (using several preparations) until reasonably consistent values were obtained.

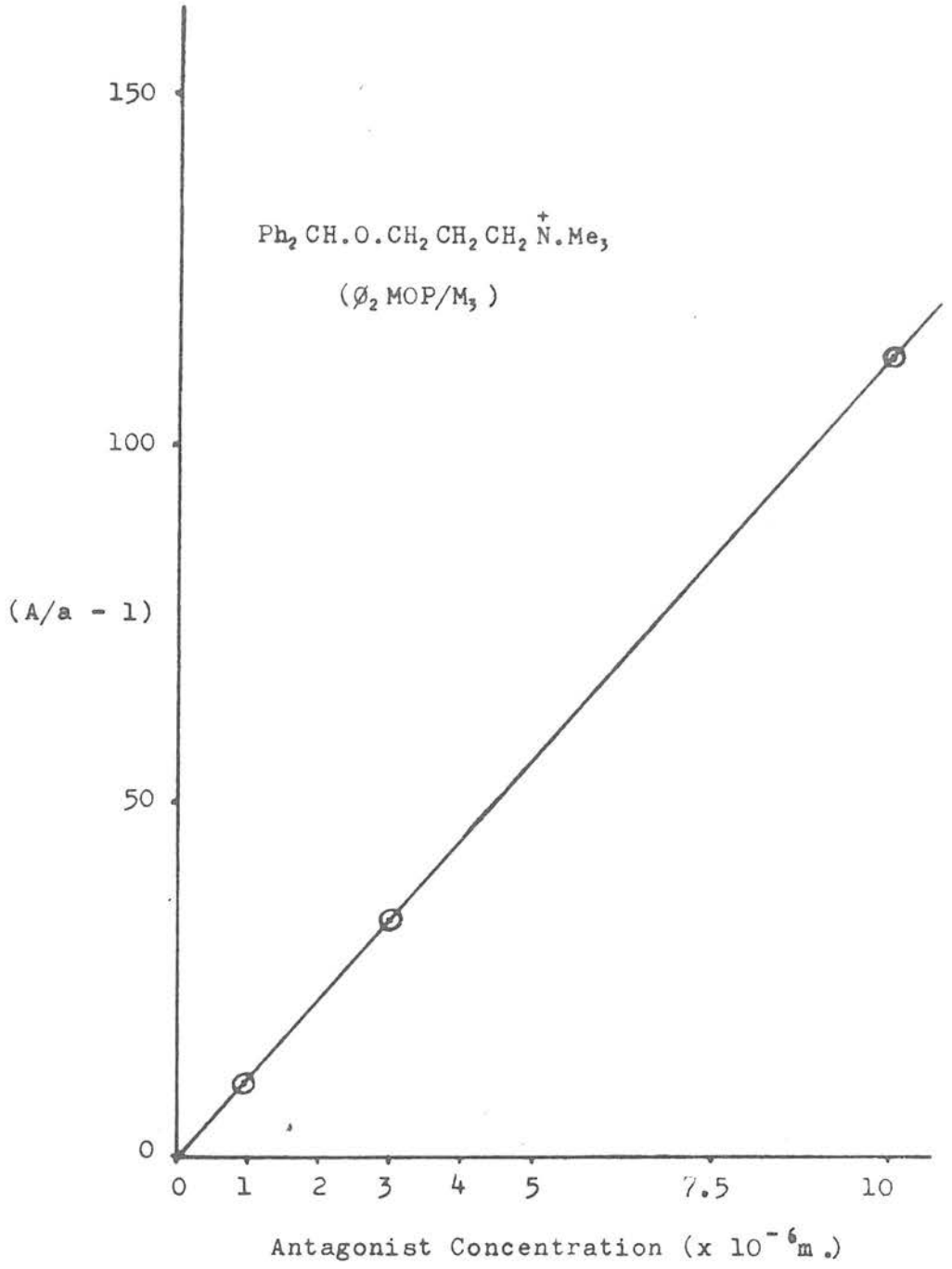


(Figure 3) This shows the method employed for obtaining the Dose-Ratios and values for the Affinity Constant from the experimental data given in Fig.2(b). A typical calculation is given below:

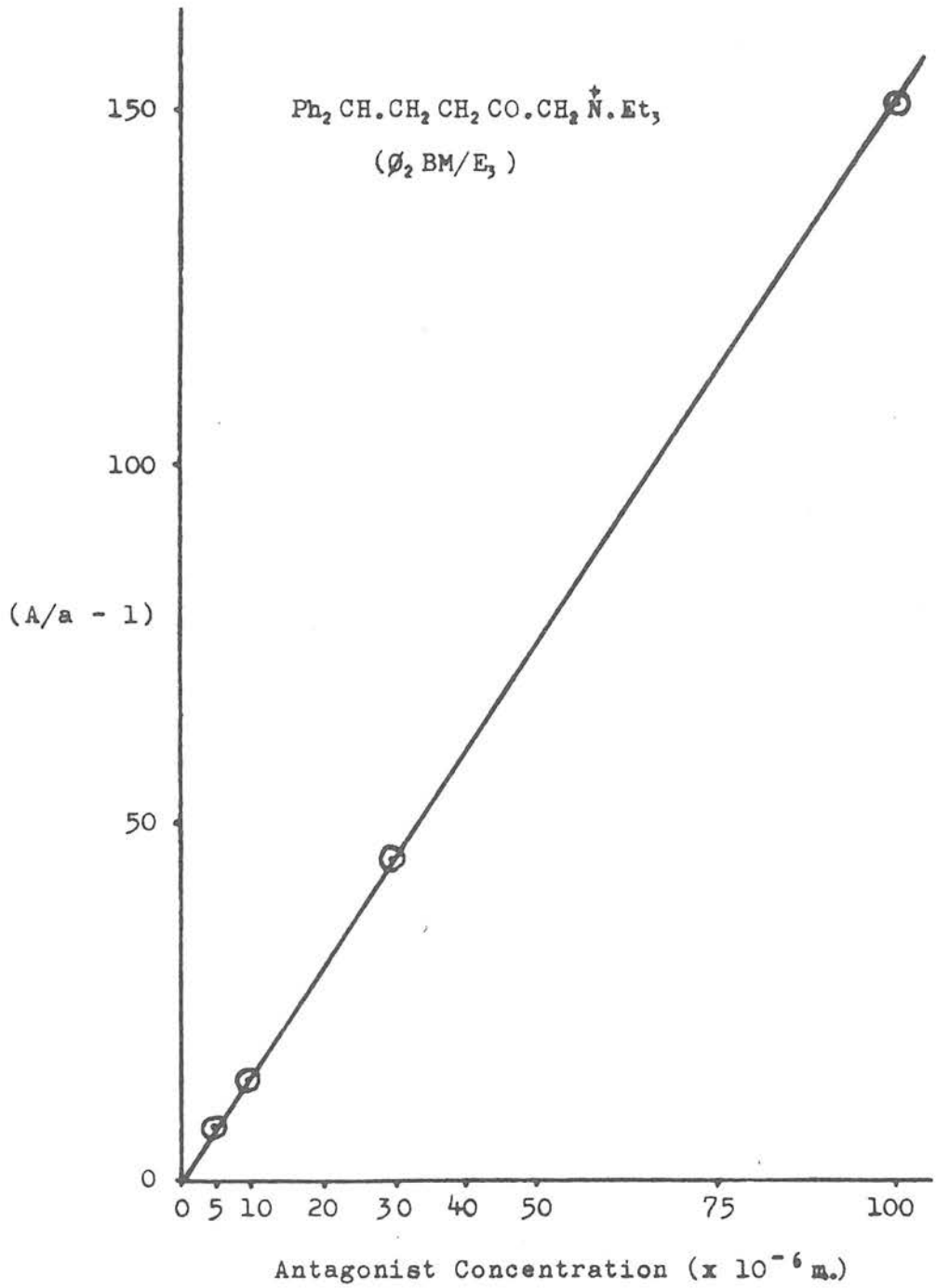
$(A/a - 1) = (80/6.58 - 1) = 11.16$

$= BK_b$ (where $B = 10^{-6}$)

Hence: $\underline{K_b = 1.12 \times 10^7}$



(Figure 4) Graph of the values of (Dose-Ratio - 1) obtained from the tracing shown in Fig.2(b) plotted against corresponding concentrations of antagonist.



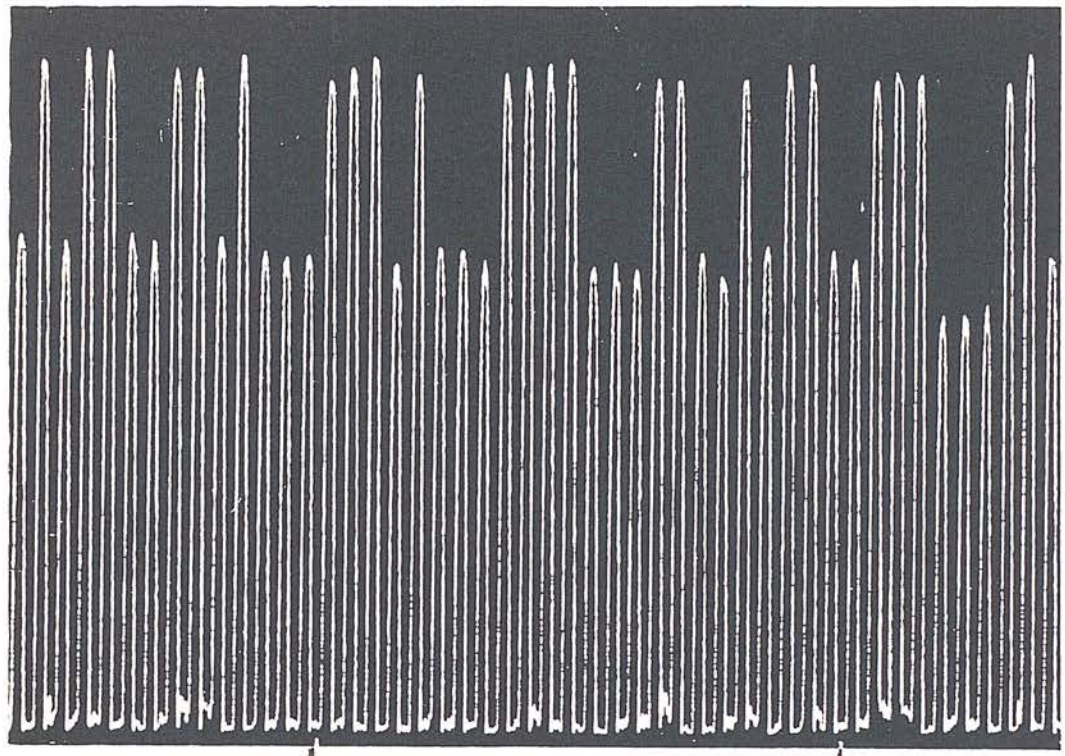
(Figure 5) Graph of (Dose-Ratio - 1) against antagonist concentration showing the linear relationship which is consistent with competitive antagonism.

Agonists.- Agonist activity was estimated by determining the equipotent molar ratios relative to acetylcholine following the procedure outlined by Stephenson (1956). Contractions of the ileum were produced either by displacing the Tyrode in the bath by a solution of the agonist in Tyrode or, as in the preliminary experiments, by the addition of agonist to the bath from a pipette at a signal from the apparatus. In addition to the uniselector controlling the cycle of operations a second uniselector was used to perform assays; 4 different agonist solutions were used in varying order to produce the contractions, after the method originally proposed by Schild (1942). Forty-eight contractions were usually employed in each assay (12 groups of 4 arranged in 3 Latin squares) (see Figure 6)

After preliminary tests had given the approximate potency of the agonist relative to acetylcholine, 2 + 2 dose assays were performed. High and low doses of acetylcholine were selected which gave contractions in the middle range of the dose-response curve. The high dose was double the low dose and the concentrations of the test agonist were chosen to give comparable contractions. In the normal 2 + 2 dose design the effect of a dose is modified by the dose immediately preceding it and this effect is sometimes quite marked. The dose order built into the equipment is so arranged that each dose is preceded by all other doses the same number of times so that this effect is not likely to bias the results but, nevertheless, the effect does increase the variation. Finney and Outhwaite (1956) have published Latin squares which are symmetrical in this way and have suggested an analysis to

eliminate the effect of the preceding dose but this analysis assumes that the effect of a dose on the following dose is independent of the size of the following dose. Experience with the guinea-pig gut has shown that this is not the case and that the interaction is more complicated; thus, for instance, a large dose will usually increase the size of contraction of a following large dose but diminish the size of contraction of a following small dose. The variance due to this cannot be eliminated by statistical treatment unless the variance due to groups (i.e. that arising from changes in sensitivity of the preparation during the course of an assay) is retained. In any case it seemed better to try to diminish the variance by modifying the experimental method. A simple procedure which was available in the design of the equipment was to interpolate a third dose of acetylcholine between each assay dose so that each assay dose, whether of acetylcholine or of test agonist was preceded by an approximately constant contraction.

The concentrations of acetylcholine used in the assays for direct comparison with the test agonist, i.e. the responses of which were actually measured, were usually 2 and 4 x 10^{-8} molar. Occasionally higher or lower concentrations were used depending on the sensitivity of the preparation. Cycles of 1 to 1½ minutes were employed with drug contact times varying from 10 to 15 seconds, with or without a second Tyrode wash. The variations were used in an attempt to obtain conditions under which regular contractions were produced and where spontaneous activity either during the rest period, or when contracted, was reduced to a minimum value.



a b c d d a c b b a d c c a b d d c b a a c d b b d c a a d b c c d a b b c a d d b a c c b d a

× y

a = Ach. 2×10^{-8} m. c = AOE/E_3 4.8×10^{-6} m.

b = Ach. 4×10^{-8} m. d = AOE/E_3 9.6×10^{-6} m.

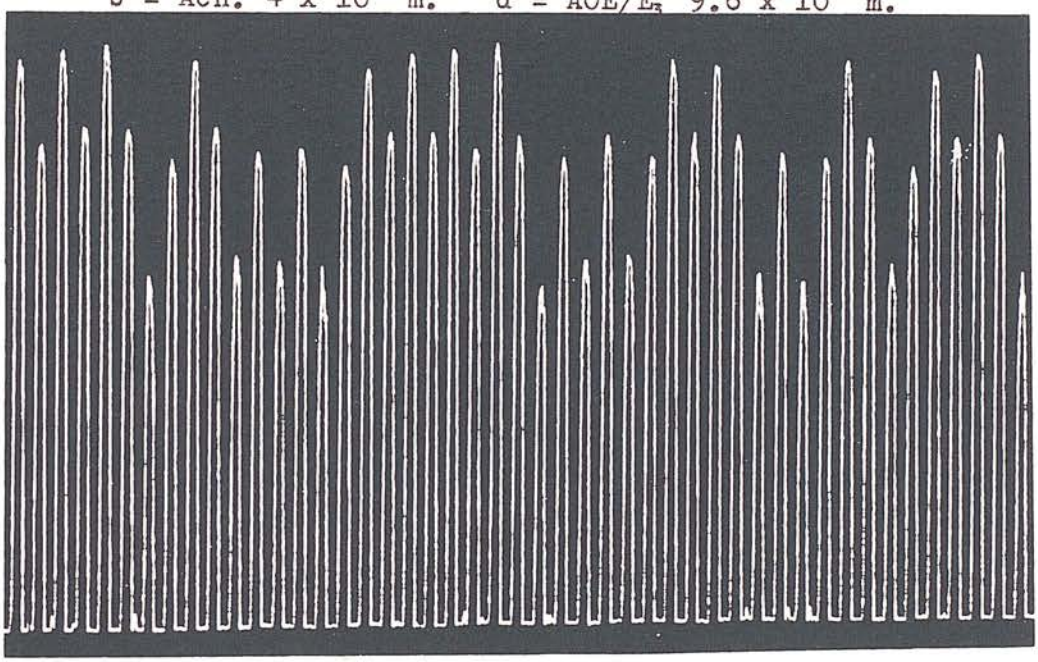


Figure 6. The upper illustration shows a complete assay of AOE/E_3 against Ach. without the usual interpolated dose of Ach. The lower illustration shows a sequence (x to y) of an assay using the same solutions in the same order on the same piece of gut, with a fixed dose of Ach interpolated between each assay dose. The effect of the preceding dose can be seen in the variation in the height of the response to the constant dose. From an analysis of variance the indices of precision (Sy/b) for the two assays were 0.01654 (interpolated) and 0.02291 (non-interpolated). Interpolated Ach. = 2.83×10^{-8} m.

Results

Activity of Antagonists: The values of the antagonist constants for the compounds are shown in Tables I to V. Estimates of K vary to a certain extent: in the most satisfactory sets of experiments the standard error was about 1% (see the results in Table V for $\emptyset_2$ EOE/M_3 and $\emptyset_2$ EOE/E_3). In the most variable determinations the error was around 10% (see the results in Table III for $\emptyset_2$ BM/ME_2 and in Table II for $\emptyset_2$ HAOE/E_3. Even in these circumstances the error compares very favourably with that observed in determinations of pA_2. For instance the results for $\emptyset_2$ BM/ME_2 (Table III) indicate a pA_2 of 6.627 ± 0.044 and for $\emptyset_2$ HAOE/E_3 (Table II), 8.676 ± 0.045. This is comparable with the error observed in estimates of pA_2 obtained by Marshall (1955) and Timms (1956) and is very much smaller than that (0.3) acknowledged by Ariëns (1960). It is particularly important that the error should be as low as possible because it affects both the numerator and denominator of the ratio k_1/k_2 which must be calculated in order to obtain the relative affinities of pairs of drugs. The means in Tables I - V are the arithmetic means and the corresponding standard error is based on the individual values of K.

In working out the ratios in Tables VI to X it is necessary to study the distribution of log. K and the means are the geometric means.

Effect of changes in structure on antagonist activity

Table I Diphenylacetyl esters ($\emptyset_2$AOE series)

$$Ph_2CH.CO.O.CH_2CH_2\overset{+}{N}.Z$$

Z	Values of K_b obtained.	Mean K_b ($\pm$S. E.)	No. of values on which mean based
Me_3	1.46×10^7 1.46 1.53	1.48 (0.02) $\times 10^7$	3
Me_2Et	4.19×10^7 4.71 4.52 4.19	4.40 (0.16) $\times 10^7$	4
$MeEt_2$	3.19×10^7 3.34 2.76 3.09	3.09 (0.12) $\times 10^7$	4
Et_3	3.00×10^7 3.28 2.90 2.80 2.28 2.72 2.68 2.92 3.10 2.30 2.36 3.52 2.26 2.14	2.73 (0.10) $\times 10^7$	14

Table II Benzilyl esters ($\emptyset_2$HAOE series)

$Ph_2C(OH).CO.O.CH_2CH_2\overset{+}{N}.Z$

Z	Values of K_b obtained.	Mean K_b (±S.E.)	No. of values on which mean based
Me_3	3.39×10^8 3.15 3.76 3.43 3.47	3.44 (0.10) $\times 10^8$	5
Me_2Et	8.00×10^8 8.12 9.05 9.04 8.84 8.89	8.66 (0.19) $\times 10^8$	6
$MeEt_2$	9.11×10^8 8.41 9.90 9.33 8.41 9.20 8.75 8.70	8.98 (0.18) $\times 10^8$	8
Et_3	4.26×10^8 5.70 4.26	4.74 (0.48) $\times 10^8$	3

Table III Diphenylpropyl ketones ($\emptyset_2$ BM series)

$Ph_2CH.CH_2CH_2CO.CH_2\overset{+}{N}.Z$

Z	Values of K_b obtained.	Mean K_b (± S.E.)	No. of values on which mean based
Me_3	1.22×10^6 1.63 1.94 1.65 2.05 1.40 1.49 1.61	1.62 (0.10) $\times 10^6$	8
Me_2Et	5.46×10^6 5.70 4.70 5.26	5.28 (0.21) $\times 10^6$	4
$MeEt_2$	3.34×10^6 3.76 4.62 5.24	4.24 (0.43) $\times 10^6$	4
Et_3	1.49×10^6 1.62 1.51 1.64	1.56 (0.04) $\times 10^6$	4

Table IV Benzhydryl ethers ($\emptyset_2$ MOP series)

$$Ph_2CH.O.CH_2CH_2CH_2\overset{+}{N}.Z$$

Z	Values of K_b obtained.	Mean K_b ($\pm$S.E.)	No. of values on which mean based
Me_3	1.12×10^7 1.16 1.14	1.14 (0.01) $\times 10^7$	3
Me_2Et	3.55×10^7 3.51 3.56 3.51 3.52 3.69	3.56 (0.08) $\times 10^7$	6
$MeEt_2$	3.76×10^7 3.75 3.39 3.50 4.08	3.70 (0.12) $\times 10^7$	5
Et_3	1.33×10^7 1.34 1.34 1.44 1.19 1.31 1.44 1.22 1.22 1.35	1.32 (0.09) $\times 10^7$	10

Table V Diphenylethyl ethers ($\emptyset_2$EOE series)

$$Ph_2CH.CH_2O.CH_2CH_2\overset{+}{N}.Z$$

Z	Values of K_b obtained.	Mean K_b ($\pm$S.E.)	No. of values on which mean based
Me_3	2.61×10^6 2.73 2.64 2.65	2.66 (0.02) $\times 10^6$	4
Me_2Et	4.56×10^6 4.32 5.78 5.47	5.03 (0.35) $\times 10^6$	4
$MeEt_2$	4.71×10^6 5.03 4.25 4.69	4.67 (0.16) $\times 10^6$	4
Et_3	3.15×10^6 3.15 3.15 3.19 3.07	3.14 (0.02) $\times 10^6$	5

Variation of Affinity with Structure

From the values of the antagonist constant, the effects of changes in structure on affinity can be calculated. These can be considered in a number of ways: if the antagonist constants for the series $R\overset{+}{N}Me_3$, $R\overset{+}{N}Me_2Et$, $R\overset{+}{N}MeEt_2$ and $R\overset{+}{N}Et_3$ are compared, the effects on affinity of altering the onium group can be studied and, since there are several series of compounds, it should be possible to see whether changes in the composition of the onium group have similar effects on affinity, regardless of the nature of R. Alternatively, by comparing the antagonist constants of $R\overset{+}{N}Me_3$ and $R'\overset{+}{N}Me_3$, the effect on affinity of altering R can be studied, since there are other compounds which differ only in the composition of the onium group, it should be possible to see whether this is the same regardless of the nature of the onium group.

In either event it is necessary to calculate the ratio K_a/K (Equation (25), page 14 where K_a is the affinity constant for the compound in which the change has been made (either replacement of methyl by ethyl or R by R' in the rest of the molecule) and K the affinity constant for the standard substance.

If a normal distribution of the values of log. K is assumed (Gaddum, 1945, 1953), the confidence limits of the ratio K_a/K can be calculated by the following procedure. The value of (log. K_a - log. K) i.e. the log. of the ratio K_a/K, will be the difference of the two mean values being studied, viz. log. $\overline{K_a}$ and $\overline{\text{log. K}}$.

The scatter of individual values of log. K_a about the mean ($\overline{\log. K_a}$) will be given by the variance $\frac{\Sigma d_1^2}{n_1 - 1}$ and the variance for the individual values of log. K from $\overline{\log. K}$ by $\frac{\Sigma d_2}{n_1 - 1}$, where ($d_1$) is the deviation of values of log. K_a from $\overline{\log. K_a}$ based on (n_1) experiments and (d_2), the corresponding values for the second set of experiments.

Estimates of the variance of results from mean values have in fact been obtained in a very large number of experiments and since this variance should have arisen from the same source in all the experiments, it is reasonable to follow the procedure of Finney (1952) and pool the estimates. The variance for individual results is then given by

$$S^2 = \frac{\Sigma d_1^2 + \Sigma d_2^2 + \cdots\cdots}{n_1 - 1 + n_2 - 1 + \cdots}$$

where the numerator is the total of the number of degrees of freedom and is numerically equal to the difference between the total number of individual experiments and the number of different drugs tested. The variance of M (where M = log. K_a/K) is given by

$$V[M] = S^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

where n_1 is the number of results on which K_a is based and n_2 the number on which K is based.

The fiducial limits of M are then obtained, again assuming normal distribution of the log. affinity constant; by direct application of the (t) distribution, (t) again being based on the total number of degrees of freedom from which the variance per result has been estimated and not simply on the number of degrees of freedom associated

with the experiments on the pair of compounds in question.

In all, 108 experiments were performed with 20 compounds (i.e. 88 degrees of freedom). The sum of the ($\sum d^2$) terms for all compounds was 0.1789 and hence the best possible estimate of the population variance is,

$$S^2 = \frac{0.1789}{88} = 0.002033$$

The following values for the affinity constants of $\emptyset_2$ AOE/M_3 and $\emptyset_2$ AOE/M_2 E may be obtained from Table I, page

$$\emptyset_2 \text{AOE}/M_2\text{E} = 4.40 \times 10^7 = K_a \quad [3 \text{ results}]$$

$$\emptyset_2 \text{AOE}/M_3 = 1.48 \times 10^7 = K \quad [4 \text{ results}]$$

Thus $\log K = 0.171$ and $\log K_a = 0.643$ [ignoring index of 10]

and $$M = \log[K_a/K] = 0.472$$

$$V(M) = S^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

$$= 0.002033(0.333 + 0.25)$$

$$= [0.03444]^2$$

for 88 degrees of freedom, t, (at the 0.05 level) has the limiting value of 1.96

$$\text{Hence, } M_L, M_U = M \pm t \quad S^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

$$= 0.472 \pm 1.96 \times 0.03444$$

$$= 0.472 \pm 0.0675$$

The values of log $[K_a/K]$ and of $[K_a/K]$ are shown in Table VI to X (summarised in Table XI) together with the 95% confidence limits, calculated as shown on page 25.
Also included in the tables are estimates, with their 95% confidence limits, of the differences in the free energies of adsorption, obtained by substitution for log $[K_a/K]$ in equation (21):

$$a = (f) = -2.3\ RT \log [K_a/K] \quad (21a)$$

$$(-f) = 1417 \log [K_a/K]$$

since, $R = 1.987$ cal. deg.$^{-1}$ mole.$^{-1}$
and $T = 37^0 C = 310^0 A.$

Effect of composition of "onium" group on affinity constant

Table VI The effect is shown as the ratio K_a/K where K is the affinity constant for the trimethylammonium compound and K_a the constant for the analogue. The value of K_a/K given in the following tables is the mean based on the experimental values listed in Tables I to V, (pages viii....), the upper and lower confidence limits, at a level of probability of 0.05, are shown in parentheses. From the logarithm of the ratio K_a/K, the value of (f) can be calculated, (f) being the change in the free energy of adsorption brought about by altering the "onium" group. In all but one case the sign of (f) is negative and for convenience therefore values of (-f) are given in the following tables.

Diphenylacetyl esters ($\emptyset_2$AOE series)

$Ph_2CH.CO.O.CH_2CH_2\overset{+}{N}.Z$

Z	K_a/K	log K_a/K	(-f)
Me_2Et	(2.54) 2.97 (3.47)	(0.405) 0.472 (0.540)	(574) 669 (765)
$MeEt_2$	(1.79) 2.08 (2.44)	(0.252) 0.319 (0.387)	(357) 452 (548)
Et_3	(1.60) 1.82 (2.08)	(0.205) 0.261 (0.317)	(290) 370 (449)

Table VII Benzilyl esters ($\emptyset_2$HAOE series)

$Ph_2C.(OH).CO.O.CH_2CH_2\overset{+}{N}.Z$

Z	K_a/K	log K_a/K	(-f)
	(2.22)	(0.347)	(492)
Me_2Et	2.52	0.401	568
	(2.92)	(0.465)	(659)
	(2.33)	(0.367)	(520)
$MeEt_2$	2.61	0.417	591
	(2.93)	(0.467)	(662)
	(1.18)	(0.071)	(101)
Et_3	1.37	0.136	193
	(1.59)	(0.201)	(285)

TableVIII Diphenylpropyl ketones ($\emptyset_2$BM series)

$Ph_2CH.CH_2CH_2CO.CH_2\overset{+}{N}.Z$

Z	K_a/K	log K_a/K	(-f)
	(2.90)	(0.462)	(655)
Me_2Et	3.28	0.516	731
	(3.72)	(0.570)	(808)
	(2.30)	(0.362)	(513)
$MeEt_2$	2.61	0.416	589
	(2.95)	(0.470)	(666)
	(0.86)	($\bar{1}$.935)	(-92)
Et_3	0.98	$\bar{1}$.989	-16
	(1.10)	(0.043)	(61)

Table IX Benzhydryl ethers ($Ø_2$MOP series)

$Ph_2CH.O.CH_2CH_2CH_2\overset{+}{N}.Z$

Z	K_a/K	log K_a/K	(-f)
Me_2Et	(2.70) 3.12 (3.61)	(0.431) 0.494 (0.557)	(611) 700 (789)
$MeEt_2$	(2.79) 3.24 (3.76)	(0.445) 0.510 (0.575)	(630) 723 (815)
Et_3	(1.01) 1.15 (1.35)	(0.004) 0.062 (0.130)	(6) 88 (184)

Table X Diphenylethyl ethers ($Ø_2$EOE series)

$Ph_2CH.CH_2O.CH_2CH_2\overset{+}{N}.Z$

Z	K_a/K	log K_a/K	(-f)
Me_2Et	(1.63) 1.88 (2.17)	(0.212) 0.274 (0.337)	(300) 388 (477)
$MeEt_2$	(1.52) 1.75 (2.03)	(0.182) 0.244 (0.307)	(258) 346 (435)
Et_3	(1.03) 1.18 (1.36)	(0.014) 0.073 (0.132)	(20) 103 (187)

Effects of composition of "onium" group on free energy of adsorption

Table XI Summary of Results: values of (-f) accompanying changes in onium group forthe series of antagonists, with confidence limits (P 0.05).

"Onium" group	Series				
	$\emptyset_2$ AOE	$\emptyset_2$ HAOE	$\emptyset_2$ BM	$\emptyset_2$ MOP	$\emptyset_2$ EOE
Me_2Et	(574) 669 (765)	(492) 568 (659)	(655) 731 (808)	(611) 700 (789)	(300) 388 (477)
$MeEt_2$	(357) 452 (548)	(520) 591 (662)	(513) 589 (666)	(630) 723 (815)	(258) 346 (435)
Et_3	(290) 370 (449)	(101) 193 (285)	(-92) -16 (61)	(6) 88 (184)	(20) 103 (187)

Justification for proceeding further

Comparison of the figures in Table XI should show whether the free energy of adsorption really can be regarded as being made up of components which are additive. Although the free energies of adsorption of the trimethylammonium compounds in the five series are all different, the change in the free energy of adsorption brought about by replacing one methyl group by ethyl should be the same, i.e. the values in the first row of figures in Table XI should be identical. Similarly the replacement of two methyl groups should produce identical values in the second row and likewise the values in the third row should be the same. There is no significant

difference ($P > 0.05$) between the values for the diphenylacetyl, ($\emptyset_2$ AOE), benzilyl ($\emptyset_2$ HAOE) and diphenylbutyrylmethyl ($\emptyset_2$ BM) series, except with the triethylammonium compounds. The values in the diphenylethoxy ($\emptyset_2$ EOE) series for the dimethylethylammonium ($\emptyset_2$ EOE/M_2 E) and the diethylmethylammonium ($\emptyset_2$ EOE/ME_2) compounds are significantly lower than those in the benzilyl, diphenylbutyryl and benzhydryl series and lower also than that for the dimethylethylammonium member ($\emptyset_2$ AOEM_2 E) of the diphenylacetyl series, but not significantly lower than the diethylmethylammonium member of the same series ($\emptyset_2$ AOE/ME_2). In the triethylammonium compounds the values for the diphenylacetyl and benzilyl compounds are significantly greater than those for the diphenylbutyryl compound.

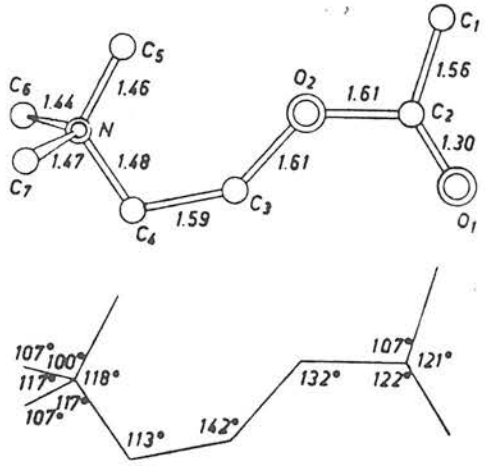
Clearly the scatter attached to the estimates of the change in the free energy of adsorption constitutes the main difficulty in the way of drawing any definite conclusion about the validity of regarding as additive the contribution to adsorption from different parts of the molecule. The values in Table XI should be compared vertically as well as horizontally to see whether in fact the experiments are capable of revealing significant differences between any of the results. In the diphenylacetyl series the value for the dimethylethylammonium compound ($\emptyset_2$ AOE/M_2 E) is significantly greater than that for the diethylmethylammonium compound ($\emptyset_2$ AOE/ME_2) but there is no significant difference between the ($MeEt_2$) and (Et_3) compounds. In all the other series there is no significant difference between the values for the (Me_2 Et) and ($MeEt_2$) compounds, within a particular series, but the value for the (Et_3) compound is invariably

smaller, and the difference is statistically significant at the 0.05 level of probability.

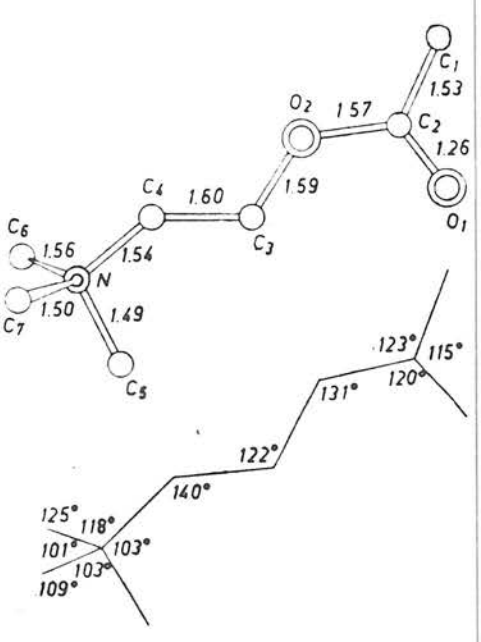
It seems possible therefore that the method employed in this work is sensitive enough to detect variations in affinity provided these are of sufficient magnitude i.e. not much less than 300 calories. However this probably represents the extreme limit of applicability of the method in its present form for the results would yield no information at all if the variance of the estimates of K were much greater than it is; for instance if it were as great as that observed by Ariëns (1960). It is impossible to conclude from the results in Table XI that the contributions of the different groups in the molecule to the free energy of adsorption are additive, yet they do not exclude such a possibility altogether. There is a general trend in the values for all five series, affinity increasing with the replacement of one or two methyl groups by ethyl and declining when the third methyl group is replaced.

There are however discrepancies, the most obvious being the positive values, shown in Table XI as negative values of (-f), in the results for the triethylammonium member of the diphenylbutyryl series ($Ø_2$ BM/E_3). These may to some extent be due to errors in experimental observations, but it seems rather unlikely that the anomaly should be due solely to inaccuracies: in this series the carbonyl group is very close to the quaternary nitrogen atom and it is conceivable that in the triethylammonium compound there is some interaction between the carbonyl with the relatively high electron density in the region of its O-atom and the large cationic head of the

molecule. With only two ethyl groups on the nitrogen atom the carbonyl group can still assume a relatively unhindered position but with the introduction of a third ethyl group this is no longer possible. Less obvious forms of interaction may also occur; for instance Sorum" (1959) has found that, in the crystal lattice, acetylcholine exists in two forms (presumably of almost identical stabilities), an extended form and a "ring" form in which the $C_\alpha - C_\beta$ bond is "skew" and not "trans" (Gill, 1958), being held in this less stable conformation by forces interacting between the ether oxygen (O_2) of the ester group and a methyl group (C_5) in the quaternary nitrogen atom, Figure below:



a. Schematic drawings of the "ring" form of the acetyl choline ion showing interatomic distances and bond angles. Interatomic distances are given in Å.



b. Schematic drawings of the extended form of the acetyl choline ion with interatomic distances in Å and bond angles.

" Sorum (1959)

In Table XI the results for the diphenylethoxy compounds are the most aberrant and it is therefore possible that in this series there exists an interaction, similar in nature to that in the esters but greater in extent due to the higher electron density which will be capable of residing on the ether oxygen in the absence of the adjacent electron-withdrawing carbonyl group, this difference may well be accentuated with the diphenylacetyl and benzilyl esters.

Whether or not this is so, the results were taken as sufficiently consistent to warrant proceeding with the second part of the work; the estimation of the effects of changes in structure on the efficacy of agonists. It was considered necessary, however, in view of the discussion above, that the changes in affinity, required for computing the changes in efficacy, should be calculated from results using the antagonist which contained similar groups to the agonist, i.e. changes in the efficacy of the esters (AOE series) were computed using values K_a/K from the two series of antagonist esters (O_2 AOE and O_2 HAOE series).

Activity of Agonists. - Values for the agonist activity expressed in equipotent molar ratios (acetylcholine = 1) are shown in Tables XII to XV. The error attached to estimates of the equipotent molar ratio appears to be very similar to that associated with the estimates of the antagonist constant. The standard error expressed as a percentage varies from 1.3% in the estimations for AOE/M_2 to 9.5% in the case of AOE/ME_2.

A number of the compounds in the agonist series were found to be partial agonists and a few behaved purely as antagonists, (see page 38).

Effects of changes in structure on efficacy

From equation (24), page 13 , the ratio of the efficacies

$$e/e_a = n.K_a/K$$

is readily obtained: the values are given in Table XVI . There are fewer results than was hoped for because many of the compounds are only partial agonists and the range of concentrations over which their dose response curves are parallel to that of acetylcholine is too small to permit an assay to be carried out. The efficacy of these, however, should be obtainable by the method of Stephenson (1956). Some of the compounds have no agonist activity and indeed behave purely as antagonists; their affinity could be measured to see if it conformed with expectation.

Effect of changes in structure on agonist activity

Table XII Acetyl esters (AOE series)

$$CH_3 CO.O.CH_2 CH_2 \overset{+}{N}.Z$$

Z	Values of (n), the equipotent molar ratio. [Ach. = 1]	Mean Value of (n), ± S.E.	No. of values on which mean is based.
$Me_2 Et$	2.94 2.78 2.80 2.81	2.84 ± 0.037	4
$MeEt_2$	354 336 436 429 397 326 387 388 370	380 ± 36	9
Et_3	367 319 347 288 266 242 235 241 240 244	275 ± 14	10

Table XIII Methyl ethers (MOP series)

$CH_3 O.CH_2 CH_2 CH_2 \overset{+}{N}.Z$

Z	Values of (n), the equipotent molar ratio [Ach. = 1]	Mean Value of (n), ± S.E.	No. of values on which mean is based.
Me_3	98 117 132 137 151 109 139	126 (± 7)	7
$Me_2 Et$	1437 1435 949 1344 1081 1065 1061 1048	1178 (± 69)	8

Table XIV Propyl ketones (BM series)

$CH_3 CH_2 CH_2 CO.CH_2 \overset{+}{N}.Z$

Z	Values of (n), the equipotent molar ratio [Ach. = 1]	Mean Value of (n), ± S.E.	No. of values on which mean is based.
Me_3	1271 1107 1100 1045	1131 (± 49)	4

Table XV Ethyl ethers (EOE series)

$CH_3 CH_2 O.CH_2 CH_2 \overset{+}{N}.Z$

Z	Values of (n), the equipotent molar ratio [Ach. = 1]	Mean Value of (n), ± S.E.	No. of values on which mean is based.
Me_3	10.6 10.1 9.8 9.0	9.9 (±0.3)	4
$Me_2 Et$	38.4 47.6 42.8 39.5 47.5	43 (±2.7)	5
$MeEt_2$	3060	-	1

Table XVI Effects of composition of "onium" group on efficacy.

Agonist	Equipotent molar ratio relative to acetylcholine	K_a/K		e/e_a	
		a	b	a	b
AOE/M_3	1.0	1.0	1.0	1.0	1.0
/M_2E	2.84	2.97	2.52	8.5	7.2
/ME_2	380	2.08	2.61	790	990
/E_3	275	1.82	1.37	510	380

Agonist	Equipotent molar ratio relative to		K_a/K	e/e_a
	acetyl-choline	trimethyl homologue		
EOE/M_3	9.9	1.0	1.0	1.0
/M_2E	43	4.3	1.88	8.1
/ME_2	3060	310	1.75	540
MOP/M_3	126	1.0	1.0	1.0
/M_2E	1180	14.3	3.12	45

Values of K_a/K are for the corresponding antagonists as shown in Tables VI to X: values in column (a) are derived from experiments with diphenylacetoxyethyl compounds ($\emptyset_2$AOE series) and those in column (b) from the benzilyloxy compounds ($\emptyset_2$HAOE).

The value of e/e_a is the ratio of the efficacy of the trimethyl compound of the particular series to that of the compound in which one or more methyl groups has been replaced by ethyl.

Discussion

Value of the Method: An assessment of the value of the method has already been made (pages 27-31): this was necessary in order to justify proceeding to study the agonists. There is, clearly, scope for improvement and indication that this could be achieved by obtaining more results. The testing, for various reasons (some mundane, some chemical), was not planned as systematically as it should have been. Although there is some doubt whether the differences in affinity revealed by the experiments with antagonists are real or not there is reason to have confidence in the inference that small changes in structure markedly decrease efficacy (Table XVI). Even if the estimates of changes in affinity derived from studies with antagonists are out by factors of two or three, the conclusion would be unaffected.

Further, although the concept of efficacy as applied by Stephenson (1956) and as used here, is not strictly applicable to Paton's theory (1961) of drug action based on the rate of occupation of receptors, the mathematical expression relating dose and response is similar in form in both theories:-

$$\text{Response} = f(S) = f(ey) = f\frac{ex}{x + 1/K} \quad \text{(Stephenson)}$$

$$\text{Response} = f\frac{k_2 x}{x + k_2/k_1} \quad \text{(Paton)}$$

where x is the dose, K, k_1 and k_2 are constants. Consequently the results are valid whichever situation is correct, the value e being replaced by k_2 if the rate of occupation of the receptors

by the drug is what leads to the biological response.

Objections may be made to the use of such a complicated structure as gut in these experiments and to the policy of treating it (or maltreating it!) with double the concentration of potassium usually present in Tyrode's solution and with Hexamethonium in order to obtain low spontaneous activity and good records. The latter procedure can, however, be defended as a measure which offsets the former criticism. In the experiments it is most unlikely that the drugs are acting at sites other than the postganglionic cholinergic receptors.

Serious criticism can also be levelled at the uncertainty about the part played by cholinesterases in modifying the actions of the drugs used in these experiments. There is evidence, however, from the work of Blaschko, Chou and Wajda (1947), that the antagonists studied are not likely to block acetylcholinesterase in the concentrations employed in this work, and from the work of Holton and Ing (1949) that those of the agonists which are esters do not differ greatly in their susceptibility to hydrolysis by the acetylcholinesterase of dog's caudate nucleus.

The most puzzling result is the relatively high agonist activity of acetoxyethyltriethylammonium: the value for the equipotent molar ratio relative to acetylcholine obtained here (275) is much less than the value (1700) recorded by Holton and Ing (1949). This point requires further investigation.

Variation of affinity with structure:-

It may be questioned whether it is not over-optimistic or naïve to suppose that the Arrhenius equation can be applied to the attachment of a drug to the hypothetical receptors in the guinea-pig ileum. The expression, however, is applied to enzymic reactions (see, for instance, Dixon and Webb, 1959) and experience in physical chemistry does not suggest any alternative. The results of this present work are, in fact, reasonable grounds for justifying its use, since they lead to estimates of changes in the free energy of adsorption which are of an order of magnitude comparable with those obtained in chemical experiments.

What is more difficult to justify is the assumption that changes in the free energy of adsorption of the antagonists with structure are applicable to the agonists (page 11). As already pointed out there is a general parallel between the values for the different series in Table XI , but there are discrepancies and some of these may be significant. It is, perhaps, fortunate that the agonists and antagonists differ in structure at a point in the molecule which is as far distant as possible from the onium group. It is also encouraging that partial agonists and even antagonists have been found in the "agonist" series, which contain a methyl group in place of a diphenylmethyl group, and consequently this point can be investigated directly.

The effect on affinity of the structure of the "body" of the molecule, i.e. whether the compounds are diphenylacetoxyethyl derivatives ($\emptyset_2$ AOE), benzilyloxyethyl derivatives ($\emptyset_2$ HAOE) etc., can be studied by comparing the relative affinities of the members of the different

series which have the same onium groups. For convenience, the members of the diphenylacetoxyethyl series were chosen as standards: the values are shown in Tables XVII - XX and summarised in Table XXI. It is a pity that the 5:5-diphenylpentyl compounds were not made, as these would be much more suitable reference substances because they do not contain a polar group.

The change in the free energy of adsorption when a hydroxyl group is introduced into the diphenylacetyl group is very considerable, being between 1.8 and 2.1 Kcals. This suggests most strongly that the hydrogen atom in the hydroxyl group is involved in a hydrogen bond with the receptor.

In the diphenylbutyrylmethyl($\emptyset_2$ BM) and diphenylethoxyethyl ($\emptyset_2$ EOE) series, the 4-carbonyl part of the diphenylacetoxyethyl group has been replaced by methylene, and the similarity between the figures for the members of these two series suggests that the 4-carbonyl group contributes between 1 and 1.5 Kcals to the free energy of adsorption and that this is not replaced by the binding of either the 3-ether oxygen atom alone or the 2-carbonyl group alone. Alternatively, the 4-carbonyl group may contribute more than 1 to 1.5 Kcals, suppose it to be 1 to (1.5 + X) Kcals, but then the 3-ether oxygen atom and the 2-carbonyl group must each contribute about the same amount (X Kcals) to offset the loss of the 4-carbonyl group. This point could be settled by the study of the 5:5-diphenylpentyl compounds.

The 4-ether oxygen atom in the diphenylmethoxypropyl ($\emptyset_2$ MOP) compounds appears

Effects of composition of the "body" of the molecule on affinity constant

Note; the term "body" denotes the major substituent on the quaternary nitrogen atom, e.g. the diphenyl-acetoxyethyl group in the molecule: $Ph_2CH.CO.O.CH_2CH_2\overset{+}{N}Z$

The effect is shown as the ratio K_z/K, where K is the affinity constant for the diphenyl-acetoxyethyl compound ($\emptyset_2$AOE) and K_z the constant for the analogue. The values have been calculated exactly as in Table

Table XVII Benzilyl esters ($\emptyset_2$HAOE series)

$Ph_2C.(OH).CO.O.CH_2CH_2\overset{+}{N}.Z$

Z	K_z/K	log K_z/K	(-f)
Me_3	(19.9) 23.2 (26.9)	(1.300) 1.365 (1.430)	(1830) 1930 (2020)
Me_2Et	(17.3) 19.7 (22.4)	(1.237) 1.294 (1.351)	(1750) 1830 (1910)
$MeEt_2$	(25.6) 29.0 (32.8)	(1.408) 1.462 (1.516)	(1990) 2060 (2140)
Et_3	(15.3) 17.4 (19.8)	(1.184) 1.240 (1.296)	(1670) 1750 (1830)

TableXVIII Diphenylpropyl ketones ($\emptyset_2$BM series)

$Ph_2CH.CH_2CH_2CO.CH_2\overset{+}{N}.Z$

Z	K_z/K	log K_z/K	(-f)
	(0.094)	($\bar{2}$.973)	(-1450)
Me_3	0.108	$\bar{1}$.034	-1360
	(0.124)	($\bar{1}$.095)	(-1280)
	(0.104)	($\bar{1}$.017)	(-1390)
Me_2Et	0.120	$\bar{1}$.079	-1300
	(0.138)	($\bar{1}$.141)	(-1210)
	(0.117)	($\bar{1}$.069)	(-1310)
$MeEt_2$	0.135	$\bar{1}$.131	-1230
	(0.156)	($\bar{1}$.193)	(-1140)
	(0.051)	($\bar{2}$.712)	(-1820)
Et_3	0.058	$\bar{2}$.762	-1750
	(0.065)	($\bar{2}$.812)	(-1680)

Table XIX Benzhydryl ethers ($\emptyset_2$ MOP series)

$Ph_2CH.O.CH_2CH_2CH_2\overset{+}{N}.Z$

Z	K_z/K	$\log K_z/K$	(-f)
	(0.652)	($\bar{1}$.814)	(-262)
Me_3	0.769	$\bar{1}$.886	-161
	(0.908)	($\bar{1}$.958)	(-59)
	(0.710)	($\bar{1}$.851)	(-210)
Me_2Et	0.809	$\bar{1}$.908	-130
	(0.923)	($\bar{1}$.965)	(-49)
	(1.04)	(0.018)	(25)
$MeEt_2$	1.19	0.077	109
	(1.37)	(0.136)	(192)
	(0.447)	($\bar{1}$.650)	(-490)
Et_3	0.486	$\bar{1}$.687	-440
	(0.530)	($\bar{1}$.724)	(-390)

Table XX Diphenylethyl ethers ($\emptyset_2$ EOE series)

$Ph_2CH.CH_2O.CH_2CH_2\overset{+}{N}.Z$

Z	K_Z/K	log K_Z/K	(-f)
	(0.153)	($\bar{1}$.185)	(-1150)
Me_3	0.179	$\bar{1}$.253	-1050
	(0.209)	($\bar{1}$.321)	(-960)
	(0.099)	($\bar{2}$.994)	(-1420)
Me_2Et	0.114	$\bar{1}$.056	-1330
	(0.131)	($\bar{1}$.118)	(-1240)
	(0.131)	($\bar{1}$.117)	(-1240)
$MeEt_2$	0.151	$\bar{1}$.179	-1160
	(0.174)	($\bar{1}$.241)	(-1070)
	(0.104)	($\bar{1}$.019)	(-1380)
Et_3	0.116	$\bar{1}$.065	-1320
	(0.129)	($\bar{1}$.111)	(-1250)

Effects of composition of the "body" of the molecule on free energy of adsorption

Table XXI **Summary of Results:** values of (-f) accompanying changes in the diphenyl-acetoxyethyl group, with confidence limits.

"Onium" group	Series			
	ϕ_2 HAOE	ϕ_2 BM	ϕ_2 MOP	ϕ_2 EOE
Me_3	(1830) 1930 (2020)	(-1450) -1360 (-1280)	(-262) -161 (-59)	(-1150) -1050 (-960)
Me_2 Et	(1750) 1830 (1910)	(-1390) -1300 (-1210)	(-210) -130 (-49)	(-1420) -1330 (-1240)
Me Et_2	(1990) 2060 (2140)	(-1310) -1230 (-1140)	(25) 109 (192)	(-1240) -1160 (-1070)
Et_3	(1670) 1750 (1830)	(-1820) -1750 (-1680)	(-490) -440 (-390)	(-1380) -1320 (-1250)

to be able largely to replace the ester link in the diphenylacetoxyethyl group. The reason for this might be that the link involves electrostatic forces between the partially negative carbonyl oxygen atom in the ester link, or the 4-ether oxygen atom, and a partially positive group in the receptor. Alternatively the oxygen atoms could be involved in hydrogen bonds but the apparently small change in free energy (1 tol.5 Kcals) observed when the 4-carbonyl group is removed does not support this idea: it would be a reasonable possibility if the real change 1 to (1.5 + X) (see above) were much bigger.

The most disturbing feature of Table XXI is the apparently difference between values obtained with different onium groups. Mostly these differences are only just significant (at the level of probability selected, P = 0.05) and can be traced to the finding that in the diphenylacetoxyethyl series the affinity is apparently maximal in the ethyldimethylammonium compound (as it is also in the diphenylbutyryl-methyl series), whereas in the benzilyloxyethyl series affinity is maximal in the methyldiethyl-ammonium compound. It is not clear why this should be, because the benzilic hydroxyl group is very far from the onium group and it would be difficult for the two to interact, particularly if the hydroxyl group is involved in a hydrogen bond. The results for the benzilic esters in these experiments run closely parallel to those obtained by Ing, Dawes and Wajda (1945) for the same compounds on the salivation and blood-pressure of the cat. On the eye, however, the ethyldimethylammonium compound, Lachesine, was more active than the methyldiethylammonium

compound. The results in the diphenylacetoxyethyl series, on the other hand, are similar to those in the diphenylbutyrylmethyl series and appear to indicate real differences.

One possible explanation for the apparent discrepancy with the benzilyloxyethyl compounds is that, because the molecule is being held more firmly at the receptor by the link through the benzilic hydroxyl group, there may be more scope for van der Waal's binding of the second ethyl group on the onium atom than is possible in the less firmly bound diphenylacetoxyethyl, diphenylbutyrylmethyl and other compounds. It may be remarked that the benzilic hydroxyl group is remarkably similar in position relative to the onium atom as is the tropic acid hydroxyl group in atropine and it would be interesting to know if the pyrrolidine bridge in atropine is comparable with the two ethyl groups in benzilyloxyethylmethyldiethylammonium.

Variation of efficacy with structure:- The results show, clearly, that efficacy is extremely dependent upon the presence of three methyl groups on the onium atom. The effects on efficacy of replacement of methyl groups on the onium atom by ethyl run parallel in the two series for which results are available. The results for acetoxyethyltriethylammonium, however, are distinctly out of line. They lead to the surprising implication that there is an increase in efficacy in proceeding from the methyldiethylammonium compound to the triethylammonium compound. Not only are these results different from those of Holton and Ing (1949), but they do not resemble those in the ethoxyethyl series of compounds, where the triethylammonium compound has such low efficacy

that it is a partial agonist. If the result is correct it may be necessary to raise some of the present concepts of the relationships between efficacy and structure, particularly the modification of Ing's ideas (1949) discussed below.

Efficacy, however, may be modified by the presence of groups in the molecule other than methyl groups on the onium atom. A 3-ether oxygen atom, in particular, appears to endow a compound with efficacy comparable with that of acetylcholine. Members of the diphenylethoxyethyl series have low affinity, comparable with that of the diphenylbutyrylmethyl compounds, yet the corresponding agonists have appreciable activity.

If the ratio K_z/K is obtained(as in Table XVII) by comparing the affinity constants of the fully methylated antagonists, $R\overset{+}{N}Me_3$, with that of diphenylacetylcholine, i.e. if it is assumed that the difference in the free energy of adsorption between the diphenylacetoxyethyl compounds and the diphenylethoxyethyl compounds is the same as that between the acetoxyethyl and the ethoxy ethyl, it is possible to estimate the relative efficacies of the compounds $R\overset{+}{N}Me_3$ and acetylcholine. Comparison can also be made of the relative efficacies of $R\overset{+}{N}Me_2Et$ and acetoxyethyldimethylethylammonium and so on. The results (Table XXII) show clearly the beneficial effects of a 3-ether oxygen atom on efficacy, regardless of whether the onium group is trimethylammonium, ethyldimethylammonium or methyldiethylammonium.

The importance of methyl groups on the onium atom for efficacy can be interpreted in a way similar to that proposed by Ing (1949) to account for their importance for activity. It may be supposed that at least two methyl groups are

Table XXII Effects of composition of the "body" of the molecule on efficacy

Agonist	Equipotent molar ratio relative to:		K_z/K	e/e_z
	acetyl-choline	AOE cpd. with same onium gp.		
EOE/M_3	9.9	-	0.18	1.8
MOP/M_3	126	-	0.77	97
BM/M_3	1130	-	0.108	120
EOE/M_2E	43	15.1	0.11	1.7
EOE/ME_2	3060	8.1	0.15	1.9
MOP/M_2E	1180	415	0.81	340

Values of K_z/K are for the antagonists with the same "onium" group but different "body": these are expressed relative to the diphenylacetoxyethyl compounds ($\emptyset_2$AOE) as shown in Table XXI .

The value of e/e_z is the ratio of the efficacy of the acetoxyethyl compound to that of the compound with the same "onium" group but with a different "body": for the compounds containing three methyl groups e is the efficacy for acetylcholine itself.

needed for the occupation by the cationic head of some hemispherical structure. The contribution of the ether group to efficacy is more difficult to interpret, but it is very interesting because it shows a clear distinction between changes in chemical structure which affect affinity and changes which affect efficacy. Once again, it would be interesting to have information about the 5:5-diphenylpentyl and n-pentyl compounds because there is the possibility that the presence of a 2-keto group of 4-ether oxygen atom may destroy efficacy, rather than that the 3-ether oxygen atom confers it.

Conclusions:- Although the results in Tables XVI and XXII, relating efficacy with structure, are rather meagre considering the work involved, this is partly because many of the compounds which might have been agonists have turned out to be partial agonists or antagonists. This work, therefore, has been of value for two reasons. Some positive results have been obtained which yield information, though not decisive information, about the ideas on which it is based. Secondly, because of those very findings which limit the information, there now appears to be the opportunity of testing the ideas by another method, namely the study of the partial agonists and antagonists found among the agonist compounds.

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Part III

CHEMISTRY

CHEMISTRY

Introduction: It was originally intended to prepare the following series of compounds:

$$X - [CH_2]_m - Y - [CH_2]_n - \overset{+}{N} \equiv Z, \quad Br^-$$

[where X = Me-, Ph_2CH-, $Ph_2C(OH)$-,

Y = -CO.O-, -CO.CH_2-, -O.CH_2-,

Z = Me_3, Me_2Et, $MeEt_2$, Et_3,

m = 0, 1 or 2, n = 2, 1 or 0,

and m + n = 2]

in the series of agonists, X was methyl and in the series of antagonists, X was benzhydryl or hydroxybenzhydryl.

Although the antagonists could often be made by a modification of the method for making the agonist, this was not always so, and in some instances the synthesis of the antagonist presented considerable difficulty.

For the pharmacological investigation it was necessary to have both the agonist and antagonist for any particular values of Y, Z, m and n: consequently, although more examples of agonists could have been prepared (some being already known in the literature), there was no point in making them because it was impossible to make the corresponding antagonists.

The general plan involved either (i) the preparation of the alkyl halide and quaternisation with

the appropriate tertiary amine or (ii) the preparation of the appropriate substituted tertiary amine and quaternisation with methyl or ethyl bromide.

Whenever possible the former route was followed since this involved the preparation of only one halide comprising the main part of the molecule. In several cases however, the second procedure proved the more convenient. In a few instances both methods were employed and this afforded a check on the structure of the final product.

Most, though not all, of the difficulties in the preparation of the antagonists could be attributed to the presence of a labile hydrogen on the benzhydryl group. No serious attempt was made to prepare the antagonists in which X was $Ph_2C(OH)$-, (other that the series $Ph_2C(OH).CO.O.CH_2CH_2\overset{+}{N}\equiv Z$,). It was felt that the synthesis of such molecules, in which at least three reactive groups would be located over a chain length of five carbon atoms, would present difficulties incommensurate with the potential value of the compounds.

Although the syntheses of the agonists were usually easier that those of the antagonists, the final products were crystallised only with difficulty and were invariably extremely hygroscopic. To some extent their tendency to absorb atmospheric moisture could be reduced by a slow recrystallisation of the pure material to give relatively large crystals with a low specific surface area.

CHEMISTRY - Experimental

AOE, $\emptyset_2$ AOE and $\emptyset_2$ HAOE series.

The final products in the above series were obtained by quaternisation of the appropriate tertiary aminoester prepared either by reaction of acyl chloride and the dialkylaminoethanol (AOE and $\emptyset_2$ AOE series) or by reaction of the dialkylaminoethyl chloride with benzilic acid, ($\emptyset_2$ HAOE series).

Diphenylacetyl chloride.

The recrystallised acid was refluxed with 1.5 molar proportions of thionyl chloride until evolution of hydrogen chloride had ceased (2 hours). after removing the excess thionyl chloride by co-distillation with benzene, the product, which boiled at 146-7° (1.3 mm.), was obtained in 86% yield: trituration with 60/80 petroleum ether gave white crystalline material m.p. 58-9°.

$R_2CH.CO.O.CH_2CH_2N.R_2'$; [R = H or Ph; R' = Me or Et].

The preparation of β-diethylaminoethyl diphenylacetate is typical of this group. Freshly redistilled diethylaminoethanol [25g.,0.213 mole] was dissolved in anhydrous ether [200 ml.] and the solution was cooled in an ice bath. An anhydrous ethereal solution of hydrogen chloride was added gradually until all the aminol had been converted to the hydrochloride which was filtered off and dissolved in chloroform [50 ml.]. Diphenylacetyl chloride [46g. 0.20 mole] was added to the solution which was then heated under reflux for 3 hours. Water was added and the aqueous layer removed, basified with ammonia solution and exhaustively extracted with ether. After drying the solution and removing the solvent, distillation of the residual oil afforded 47.3g. of product $b_{0.1}$ 164-164.5°, and $n_D^{17.5}$ 1.5396. Yields and characteristics of all members of the group are given in Table 1 overleaf.

Table 1 $R_2CH.CO.O.CH_2CH_2N.R'_2$

R	R'	Yield(%)	b.p.(mm.Hg)	n_D (temp.)
H	Me	62	151° (760)	1.4180 (17.5°)
H	Et	74	180-1° (760)	1.4254 (17.5°)
Ph	Me	66	213° (15.0)	1.5468 (22.5°)
Ph	Et	76	164° (0.10)	1.5396 (17.5°)

$Ph_2C(OH).CO.O.CH_2CH_2N.Me_2$ The method used for the synthesis of this compound and of the corresponding diethylamino analogue was essentially that of Horenstein and Pählicke (1938) as modified by Burtner and Cusic (1943). A mixture of benzilic acid [14.2g.,0.062 mole], dimethylaminoethyl chloride [7.0g., 0.065 mole] dissolved in isopropanol [50 ml.] was heated under reflux for 2 hr. Anhydrous ether was added to the warm solution until just short of a permanent turbidity: the hydrochloride of the product crystallised slowly from the solution, 17.0g. m.p. 184-5° being obtained. This represents a yield of 81% theory; with the diethylamino ester the yield of hydrochloride m.p. 177-8° was 77%.

The bases were extracted from conc. solutions of their hydrochlorides, after adding alkali, and, after removing the solvent, the amino-esters were converted into the quaternary compounds without further purification.

The quaternary ammonium compounds were prepared by reacting the tertiary aminoester with a twofold excess of methyl or ethyl bromide in anhydrous butan-2-one. The reaction mixture was in each case allowed to stand for two days at room temperature and this was followed by a brief period of reflux to ensure completion of the reaction. Although by the end of this time most of the product had crystallised from the solution, anhydrous ether was added until just short of a permanent turbidity to obtain the maximum possible yield. Details of the products are given in Table 2.

Table 2 $R_2 \underset{R'''}{C}.CO.O.CH_2CH_2\overset{+}{\underset{R''}{N}}.R_2' \quad Br^-$

R	R'	R''	R'''	Yield of pure material	m.p.	Solvent for crystn.
H	Me	Me	H	.....acetylcholine.....		
H	Me	Et	H	53%	95°	Me_2CO/EtOAc
H	Et	Me	H	62	62	
H	Et	Et	H	75	134	
Ph	Me	Me	H	89	201	EtOH/EtOAc
Ph	Me	Et	H	85	150	
Ph	Et	Me	H	87	108	
Ph	Et	Et	H	82	154	
Ph	Me	Me	OH	70	187[1]	EtOAc-EtOH/Et_2O
Ph	Me	Et	OH	81	178	
Ph	Et	Me	OH	90	176[2]	
Ph	Et	Et	OH	93	226[3]	

[1] Previously reported by Ford-Moore and Ing (1947) as the chloride.

[2] Blicke and Maxwell (1942) give 169-70°.

[3] Ford-Moore and Ing (1947) give 200°.

Table 3 Analytical Results

Note: Analysis of the quaternary ammonium compounds was restricted to the volumetric determination of ionic bromine except in those cases where the characteristics of the immediate precursor of the "quaternary" were not recorded in the literature.

Compound	Formula	Molec. Weight	% ionic Br	
			Found	Required
AOE/M_2 E	$C_8H_{18}O_2NBr$	240.1	33.00	33.29
AOE/ME_2	$C_9H_{20}O_2NBr$	254.2	31.49	31.44
AOE/E_3	$C_{10}H_{22}O_2NBr$	268.2	30.01	29.80
$Ø_2$ AOE/M_3	$C_{19}H_{24}O_2NBr$	378.3	21.02	21.13
$Ø_2$ AOE/M_2 E	$C_{20}H_{26}O_2NBr$	392.3	20.37	20.37
$Ø_2$ AOE/ME_2	$C_{21}H_{28}O_2NBr$	406.3	19.50	19.67
$Ø_2$ AOE/E_3	$C_{22}H_{30}O_2NBr$	420.4	18.83	19.01
$Ø_2$ HAOE/M_3	$C_{19}H_{24}O_3NBr$	394.3	20.02	20.27
$Ø_2$ HAOE/M_2 E	$C_{20}H_{26}O_3NBr$	408.3	19.62	19.57
$Ø_2$ HAOE/ME_2	$C_{21}H_{28}O_3NBr$	422.4	18.80	18.92
$Ø_2$ HAOE/E_3	$C_{22}H_{30}O_3NBr$	436.4	18.34	18.31

BM and $\emptyset_2$BM series.

In both series the appropriate acyl chloride was converted, via the diazoketone, into the acylmethyl bromide by reaction with diazomethane followed by treatment with hydrobromic acid.

Butyrylmethyl bromide.

An ethereal solution of diazomethane was prepared by the portionwise addition of N-nitroso-methylurea [40g.,0.388 mole] to a vigorously-shaken suspension of 40% ($^{w}/_{v}$) potassium hydroxide solution [120 ml.] in ether [250 ml.]. The ethereal solution so obtained was dried over potassium hydroxide pellets for several hours at -25°. Redistilled butyryl chloride [b_{760} 102°, 13.3g.,0.125 mole] dissolved in ether [50 ml.] was added dropwise, over a period of 20 minutes, to the diazomethane solution which was continuously shaken in an ice-water bath. After an initial delay nitrogen was vigorously evolved. The reaction mixture was then concentrated, and the excess diazomethane removed, by passing a current of dry air over the liquid. Hydrobromic acid [60%, S.G. 1.7, 11.2 ml.] was then carefully added to the concentrated ethereal solution of the diazoketone; again nitrogen was evolved. The addition of acid was continued until effervescence ceased. The aqueous layer was removed and the ether layer was washed with 10% sodium bicarbonate solution, and dried over sodium sulphate. The solvent was removed and the residual oil was distilled to yield 12.7g. of a clear water-white liquid $b_{2\cdot0}$ 37-8° and having n_D^{20} 1.4573. The yield amounted to 62% of the theoretical. A second preparation afforded approx. the same yield of material b_{18} 70-1°

2:2-Diphenylpropionic Acid.

This acid was prepared by the method of Pfeiffer and de Waal (1935) although a different

method of extracting the product from the reaction mixture was employed. To solution of cinnamic acid [100g.,0.68 mole] in benzene [1.2 litre] finely-powdered aluminium chloride [I.C.I.,40g.,0.30 mole] was added with constant stirring, the temperature of the reaction being maintained at 10-15° throughout the addition. After about one half of the aluminium chloride had been added the temperature of the solution showed a tendency to rise and at the same time evolution of hydrogen chloride started. The mixture was allowed to stand overnight at room temperature and then more $AlCl_3$ [160g.,1.20 mole] was added and again the mixture stood overnight. The highly-coloured $AlCl_3$-complex was decomposed by pouring into ice-water containing conc. hydrochloric acid [400 ml.]. The solution was extracted with ether and the benzene/ether solution so obtained was extracted with sodium hydroxide solution. The alkaline extract was acidified and the precipitated acid was removed in a basket centrifuge and spun dry: 148g. of air-dried material m.p.150-2° was obtained. This was dissolved in boiling ethanol [250 ml.] and water [125 ml.] was added: pure diphenylpropionic acid crystallised from the solution; 133g. melting at 154° were obtained, (yield = 87% theoretical).

3:3-Diphenylbutyric Acid.

This acid, which was first reported by Ziegler (1929), was prepared more recently by Dippy and Young (1955) by a Stobbe condensation between diethyl succinate and benzophenone in 39% yield. The latter authors claim that "a much poorer yield was given by the Arndt-Eistert process". As there appeared to be no reason for supposing, on theoretical grounds, that this should be the case, it was decided to attempt the preparation of the acid from the readily-available diphenylpropionic acid by the Arndt-Eistert reaction.

2:2-Diphenylpropionic acid [45.2g., 0.20 mole] was dissolved in ether [100 ml.] and thionyl chloride [21.5ml.= 36g.,0.30 mole] and a few drops of pyridine were added. After the initial reaction had subsided the solution was heated under reflux for 3 hours. The solvent and excess thionyl chloride were removed by distillation and the crude acyl chloride was fractionally distilled from an air-bath to yield 41.6g (85% theoretical) of a colourless slightly -viscous liquid $b_{0.035}$ 126° which rapidly crystallised to a white solid m.p.140-1°.

An ethereal solution of diazomethane was prepared as previously described from N-nitroso-methylurea [90.0g.,0.875 mole] and 40% potassium hydroxide solution. To this was added, dropwise over a period of 20 minutes, a solution of 2:2-diphenyl propionyl chloride [32.0g.,0.132 mole] in ether [100ml]. A vigorous evolution of nitrogen took place almost immediately the addition was started and the colour of the diazomethane solution faded until a pale yellow. The reaction mixture was allowed to stand overnight at -25° during which time large elongated prisms of the yellow diazoketone separated from the solution. The ether was removed by distillation under reduced pressure from a water-bath at 30° to yield the diazoketone as a highly-crystalline mass. A small sample of the diazoketone was recrystallised from ethanol to give stout prisms melting at 78-9°.

The remainder of the diazoketone was dissolved in methanol [250 ml.] and to it was added a slurry of silver oxide (from 10 ml. 10% aq.$AgNO_3$ and an excess of NaOH, water removed by repeated washing with methanol) in methanol [50 ml.] The mixture was slowly heated on a water-bath: at 40° a vigorous evolution of nitrogen commenced and continued as the temperature was raised to 50-55° and thereafter for about 20 minutes after which it

subsided. The addition of a further quantity of silver oxide (equivalent to 5 ml. 10% $AgNO_3$) caused more nitrogen to be evolved and, about this time, a silver "mirror" started to form on the wall of the flask. After 20-30 minutes heating (50-55°) more silver oxide (equivalent to 5 ml. 10% $AgNO_3$) was added and the mixture was heated under reflux for 1 hour. Completion of reaction was shown by the failure to produce evolution of nitrogen on adding a little conc. HCl to a small sample of the reaction mixture. After filtration, the solvent was removed and the residual ester was distilled to yield 30.7g. (97.5% theory) of a colourless limpid liquid b 147.5-148° having n_D^{22} 1.5575 and D_4^{22} 1.082. The calculated value for the Molar Refraction was 79.90 which corresponded closely with the observed value of 79.78.

The ester [25g., 0.0985 mole] was hydrolysed by refluxing for 4 hours with a solution of sodium hydroxide [10g.] in water [60 ml.]. The resulting clear solution was diluted with water, extracted with ether and added to a cooled solution of HCl. An oil was precipitated which solidified on standing to give 21.4g. crude acid m.p. 102-3°. Crystallisation from aqueous ethanol gave 19.3g. (81.5% theory) of acid m.p. 105°, which is the value recorded by Ziegler (1929) and Dippy and Young (1955). The overall yield for the process, based on diphenylpropionic acid amounted to 67.5% . The overall yield obtained by Dippy and Young, calculated from their reported results, amounts to 39% of the theoretical. It would therefore appear that the Arndt-Eistert synthesis of the acid is, in fact, preferable to that involving a Stobbe condensation.

3:3-Diphenylbutyrylmethyl bromide was prepared in 79% of the theoretical yield from the acid by a procedure identical with that already described for butyrylmethyl bromide. The product, which crystallised from ethanol in fine needles, (m.p. 95°), gave the following values on analysis:

	Carbon %	Hydrogen %
Found	64.70	5.33
Required for $C_{17}H_{17}BrO$	64.37	5.40

Quaternary Ammonium Compounds

As might reasonably be expected the reaction of butyrylmethyl bromide and the corresponding diphenyl compound with the tertiary amines in butan-2-one proceeded very rapidly to give almost quantitative yields of products. Previous experience with acylmethyl bromides had shown that under certain circumstances quaternisation was accompanied by a secondary reaction involving either intra- or inter- molecular elimination of HBr. This secondary reaction had been found to proceed to a considerable extent where there was present in the acyl group, a labile hydrogen, other than on either carbon adjacent to the carbonyl group. Such a situation seemed to offer the best conditions for the intra-molecular elimination of HBr which is intrinsically easier to achieve than the inter-molecular reaction. If a reaction of this nature did occur the product would consist of a mixture of quaternary ammonium salt and the acid salt of the tertiary amine. Such a mixture is exceedingly difficult to separate by physical means and i separation is best achieved by an adaption of the method of Sackur (193) in which a methanolic solution of the mixed product is treated with propylene oxide and the salt-free quaternary is precipitated from solution by the addition of ether.

Diphenylbutyrylmethylmethyl bromide is capable of the ready elimination of HBr due to the labile hydrogen present in the terminal benzhydryl group. The recrystallisation of the quaternary compounds derived from this bromide was therefore carried out with the addition of a little propylene oxide to a concentrated ethanolic solution of the quaternary. After standing, the solution was warmed and hot ethyl acetate added until crystallisation started.

Table 4 Yields and characteristics of products:

$R_2CH.CH_2CH_2CO.CH_2\underset{R''}{N}.R'_2$

R	R'	R''	Yield %	m.p.	ionic Bromine % found	ionic Bromine % calculated
H	Me	Me	90	145°	35.43	35.68
H	Me	Et	82	109	33.55	33.60
H	Et	Me	87	103	31.85	31.76
H	Et	Et	72	194	30.00	30.05
Ph	Me	Me	93	137	21.36	21.24
Ph	Me	Et	92	123	20.30	20.46
Ph	Et	Me	87	127	19.70	19.77
Ph	Et	Et	87	169	19.18	19.10

MOP and $\emptyset_2$ MOP series

Preparation of the amino-ethers followed by quaternisation, gave the members of both series.

3:3-Diethylaminopropan-1-ol

Following the method of Munch, Thaunhauser and Cotile (1946) trimethylene chlorohydrin, diethylamine and sodium bicarbonate in the molar ratio of 1 : 3 : 1.5 were heated together under reflux in solution in 50% aqueous alcohol. A 78% yield of the product was obtained $b_{15\cdot0}$ 83°, n_D^{20} 1.4442, D_4^{20} 0.8808

[M.R.] obs. 39.58 calcd. 39.30.

$CH_3OCH_2CH_2CH_2NR_2$ [R = Me or Et]

These amino-ethers were prepared by reacting the dialkylaminopropyl chloride with sodium methoxide in benzene solution.

To a solution of methanol [3.5 g. 0.11 mole] in anhydrous benzene [75 ml.] sodamine [4.25 g. 100% 0.11 mole] was added. The mixture was refluxed with stirring until evolution of ammonia had ceased (c. 1 hour). A benzene solution of the dialkylaminopropyl chloride, obtained by extraction of a concentrated aqueous solution of the hydrochloride after basification with K_2CO_3, was added to the cooled mixture which was then heated under reflux for a further 4 hours, during which time sodium chloride was precipitated. The mixture was cooled, filtered and the benzene removed by distillation. Fractionation of the residual oil gave the desired product.

The corresponding benshydryl compounds

were obtained in a similar manner although a slightly different extraction method was employed to remove chemically any traces of unchanged benzhydrol. Water was added at the end of the reaction and after discarding the aqueous layer, the benzene layer was extracted with 2N HCl; the extract was washed with ether, basified with ammonia and extracted with ether. The combined extracts were dried and after removal of solvent the residue was fractionally distilled. The yields and characteristics of the products are given in Table 5 below.

Table 5 $R_2CH.O.CH_2CH_2CH_2NR_2'$

R	R'	Yield (%)	b.p. (mm.Hg)	n_D^{20}
H	Me	69	128-9(762)	1.4270
H	Et	76	166-7(762)	1.4403
Ph	Me	85	190-1(10.0)	1.5425
Ph	Et	82	208-9(11.0)	1.5341

Quaternary Ammonium Compounds

Yields and characteristics of the quaternary ammonium compounds which were prepared in the usual manner are collected in Table 6.

Table 6. $R_2CH.O.CH_2CH_2CH_2N.R_2'$ (R'' = R'')

R	R'	R''	Yield %	m.p.	ionic Bromine %	
					found	calculated
H	Me	Me	67	230°	41.40	41.50
H	Me	Et	71	167	36.01	35.43
H	Et	Me	53	185	33.30	33.34
H	Et	Et	78	140	31.27	31.47
Ph	Me	Me	93	180	21.95	21.95
Ph	Me	Et	86	129	21.47	21.20
Ph	Et	Me	86	112	20.40	20.40
Ph	Et	Et	90	157	20.01	19.71

Those compounds in which R = H were crystallised from acetone/ethyl acetate and those in which R = Ph from ethanol/ethyl acetate.

EOE and $\emptyset_2$EOE series.

Both series were obtained by quaternisation of the amino-ethers which were prepared by the method of Protiva (1954).

$Ph_2CH.CH_2O.CH_2CH_2N.R_2$; [R = Me or Et].

A solution of 2:2-diphenylethanol [19.8g.,0.10 mole, m.p.61°] in anhydrous benzene [50ml. sodium dried] was added rapidly to a stirred suspension of sodamide [5.0g.,0.13 mole] in benzene [50 ml.]. After the initially rapid evolution of ammonia had subsided, the mixture was heated under reflux with constant stirring for 1 hour. A solution of 2-dimethylaminoethyl chloride (extracted from the hydrochloride [18g.,0.125 mole] in the usual manner) in benzene [60 ml.] was then added, over 2 minutes, to the cooled reaction mixture. A mildly exothermic reaction ensued and once this had subsided the solution was refluxed for four hours. At the end of this time a considerable deposit of sodium chloride was found in the flask. Water [100 ml.] was carefully added to the stirred and cooled mixture: the aqueous layer was removed and discarded after washing with ether. The benzene layer, together with the ether washings, was extracted with 2N HCl. The acid extract was basified with ammonia and the product extracted into ether. After removing the solvent the crude amino-ether was fractionally distilled to give 22.6g of a clear liquid $b_{0.05}$ 155-6° .

This boiling point differed markedly from that reported by Protiva (1954) for the same compound, viz. $b_{1.0}$ 143-4° : no other characteristics of the substance were given by him. In order to verify that the correct compound had in fact been obtained, the Molar Refraction was determined and was found to be in close agreement with the value required by theory.

The yields and characteristics of the dimethylamino ether and of the diethylamino analogue which was prepared in a similar manner are given in Table 7 along with the data for the two ethers of the EOE series.

<u>$CH_3 CH_2 O.CH_2 CH_2 N.R_2$; [R = Me or Et].</u>

These ethers were prepared by reacting the sodio derivative of the appropriate amino-alcohol with ethyl bromide in benzene solution. The reaction was much more vigorous than for the diphenyl compounds and, in addition, the relatively high water solubility of the substances made extraction from the acid solution much more difficult even after saturation of the solution with solid potassium carbonate.

Table 7 $R_2 CHCH_2 O.CH_2 CH_2 N.R_2'$

	R = H R' = Me	R = H R' = Et	R = Ph R' = Me	R = Ph R' = Et
Yield(%)	49	62	84	91
b.p.(mm.)	120° (756)	154° (756)	156° (0.05)	169° (0.4)
n_D^{18}	1.4060	1.4181	1.5475	1.5385
D_4^{18}	0.8067	0.8137	1.012	0.9996
[M.R.] obs.	35.68	44.98	84.42	93.14
[M.R.] calc.	35.49	44.73	84.70	92.94

Quaternary Ammonium Compounds

A mixture of solvents was employed for the quaternisation reactions as most of the products were found to be too soluble in butanone (M.E.K.) alone. The amino-ether [0.03 mole] was dissolved in anhydrous M.E.K. [15 ml.] and to the solution was added the alkyl halide [0.05 mole]. After about 24 hours, ethyl acetate was slowly added until just short of a permanent turbidity. The solution was then left at room temperature for 3-4 days. The yields and characteristics of the products are collected in Table 8.

Table 8 $R_2CH.CH_2O.CH_2CH_2N.R_2'$ (R'')

R	R'	R''	Yield %	m.p.	ionic Bromine %	
					found	calculated
H	Me	Me	78	183°	41.42	41.50
H	Me	Et	44	69	35.33	35.43
H	Et	Me	69	58++	33.61	33.34
H	Et	Et	56	97	31.54	31.57
Hh	Me	Me	94	147	22.10	21.95
Ph	Me	Et	78	106	21.13	21.20
Ph	Et	Me	90	128	20.37	20.40
Ph	Et	Et	90	167	19.80	19.71

++ Iodide.

The solvents used for recrystallisation were acetone/ethyl acetate in those compounds in which R = H, and ethanol/ethyl acetate where R = Ph.

Incomplete Series.

In addition to the five antagonist and four agonist series attempts were made to prepare several other series. The propoxymethyl series of "agonist" was prepared ($CH_3 CH_2 CH_2 O. CH_2 \overset{+}{N} Z$) from the aminoethers themselves obtained from propan-1-ol by the method of Stewart and Bradley (1932). Preparation of the corresponding antagonist series proved very troublesome although the first two members of the series were obtained from 3:3 diphenylpropoxymethyl bromide obtained from 3:3 diphenylpropanol by a modification of the method described by Henze, Duff, Matthews, Melton and Forman (1942). This bromide proved to be exceedingly unstable and crystalline quaternary salts giving the required values for C, H and Br on analysis, could be obtained only in the case of the trimethylammonium and dimethylethylammonium compounds.

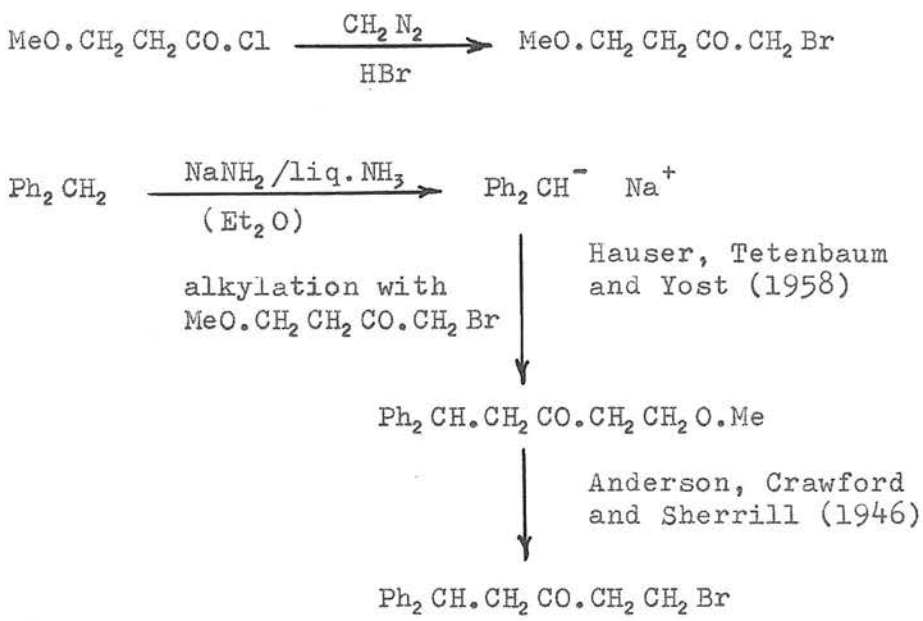
Most disappointing of all was the failure to produce the antagonist series of ketones with the carbonyl groups in position 3. It is, of course, impossible to utilise the method of Cardwell and McQuillin (1949) employed by Ing, Kordik and Tudor Williams (1952) (for the preparation of 1-chloropentan-3-one) in this case due to the Friedel-Crafts type self-acylation of the diphenylpropionyl chloride in the presence of the aluminium chloride. Various modifications of the method of Cason (1946) were used in attempts to prepare the cadmium derivative of 2:2 diphenylethyl bromide for subsequent reaction with methoxy-propionyl chloride, but, even when using a "cyclic reactor" similar to that described by Rowlands, Greenlee and Boord (1950), a negative Gilman test for the presence of a Grignard reagent was invariably obtained. The only product recovered (in high yield.) from the

reaction mixture was identified as :- $Ph_2C=C.\overset{Ph}{\underset{Ph}{C}}.CH_3$

Although it was formerly held that the precursors of $Ph_2CH.CH_2CO.CH_2CH_2Br$ were too unstable to allow the bromoketone to be prepared it now appears that the substance could be obtained by a direct alkylation of diphenylmethane after the method of Hauser, Tetenbaum and Yost (1958) a proposed reaction scheme is given on page 62.

Proposed Reaction Scheme for the Synthesis of

1-Bromo-5:5-diphenylpentan-3-one



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